

# The Basic Science Behind the Potent New Immunotherapies

iSBT

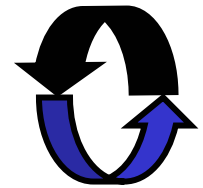
November 11, 2005

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Principal Investigator



## Goal: To develop new immunotherapies for patients with cancer

- Strategy: create new animal models that accurately represent the treatment of humans with established tumors.
- Using an iterative process, continually translate bench research into new clinical trials.
- Model clinically generated hypotheses back into the mouse.



## A view of translational research

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The assumption is that mice and humans are evolutionarily close enough so that findings in one species can be translated into the other.

The challenge is finding realistic mouse models that emulate the human experience.

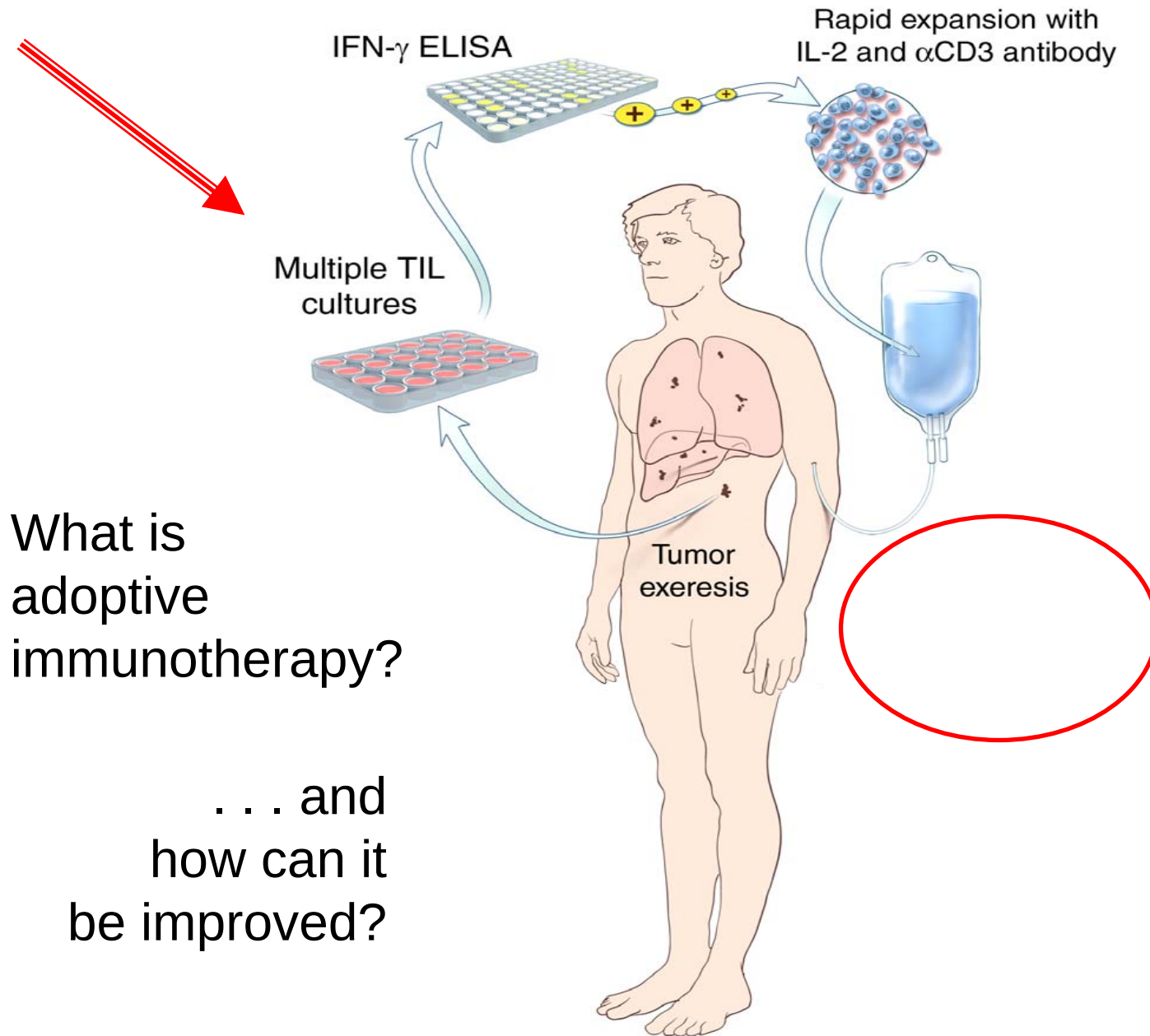
# The challenge: Treat established, vascularized solid tumors in normal mice



## *The rules:*

Tumor must be unmanipulated -- no artificial insertion of costimulatory molecules, alloantigens or neoantigens [like molecules from humans, chickens, bacteria or viruses].

Treatment schema must be realistic -- must be suitable for metastatic (systemic) disease. Cannot involve the manipulation of the mouse prior to its “presentation” to the mouse clinic with a large tumor.



# **Lymphodepletion followed by adoptive cell transfer induces objective responses in half of the patients**

**13 Patients → 6 Objective Responses (45%)**

*Dudley, Rosenberg, Science 2002*

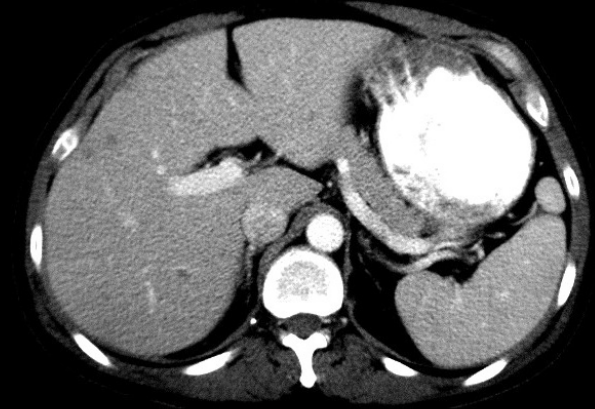
**35 Patients → 18 Objective Responses (51%)**

*Dudley, Rosenberg, JCO 2005*

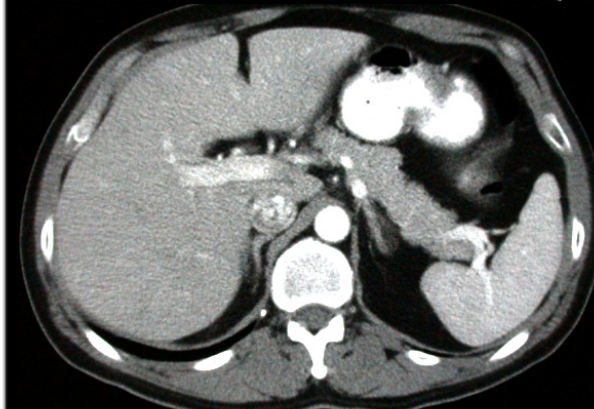
**Pt.R.B.**



**Day -25**



**Day +34**



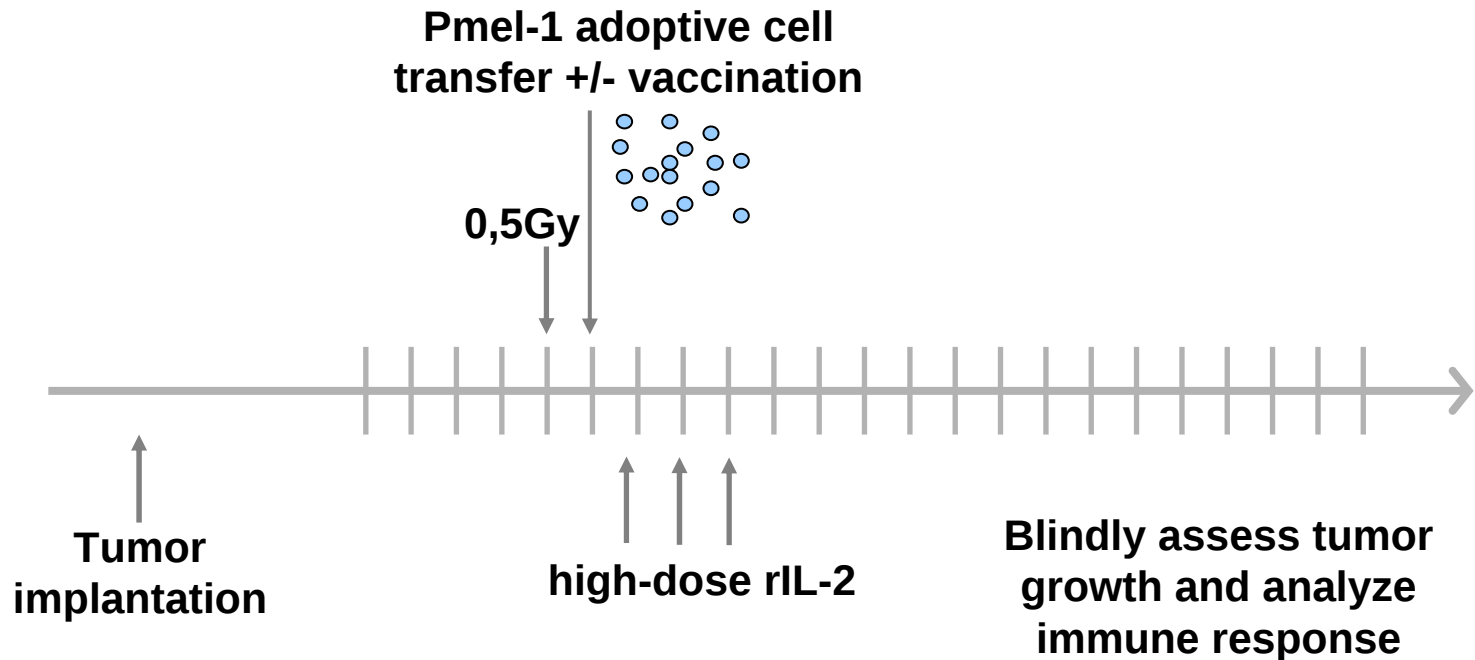
**18+ Months**

# Modeling the impact of lymphodepletion in the mouse

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- It has long been observed that the administration of cyclophosphamide or total body irradiation (TBI) could augment the activities of T cell-based immunotherapies.
- We sought to explore the impact of irradiation and chemotherapy in the induction of a lymphopenic host environment.
- We wanted to understand if these modalities could be used to enhance T cell-based treatment in the pmel-1 model.

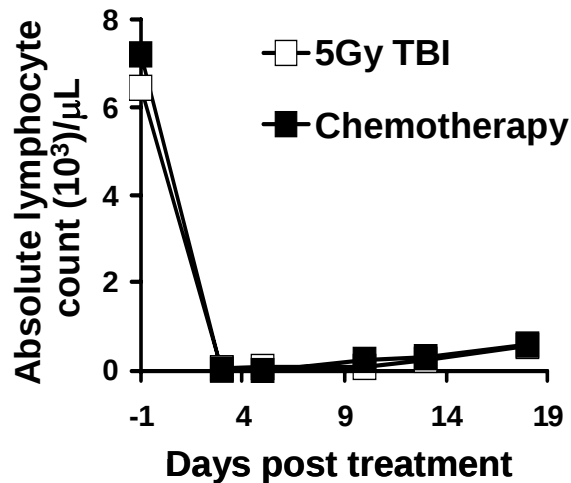
# Pmel-1 ACT murine melanoma model



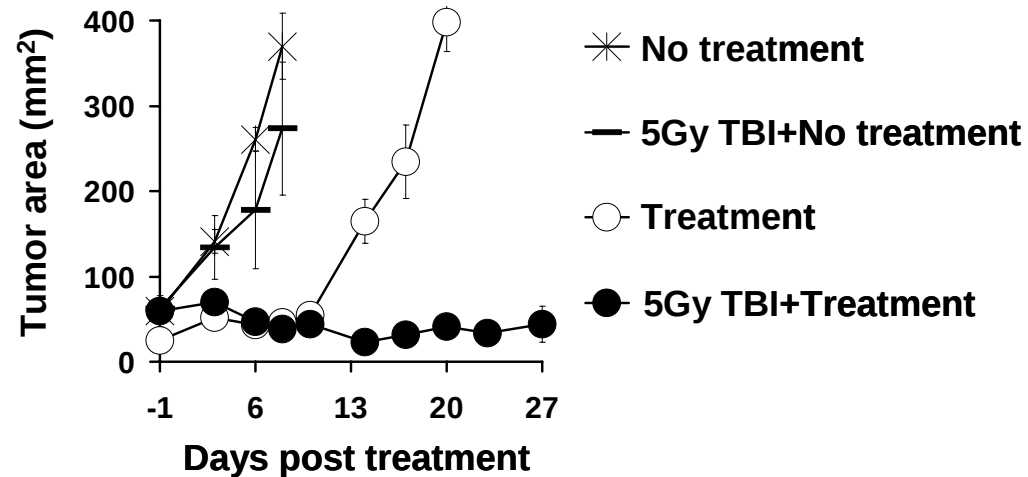
*Overwijk et al. J exp med 2003*

# Lymphodepletion enhances the anti-tumor efficacy of adoptively transferred CD8<sup>+</sup> T cells

a



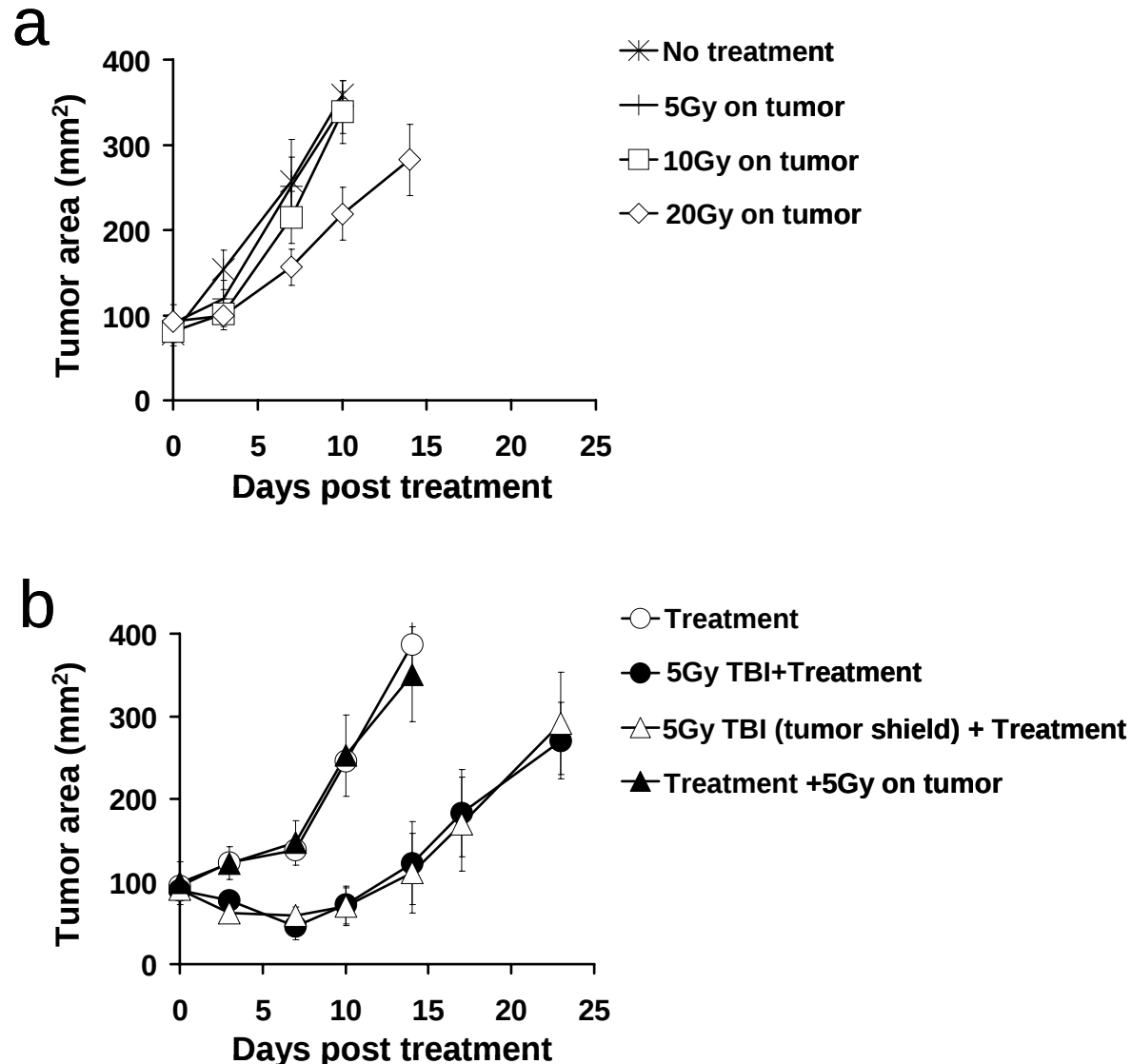
b



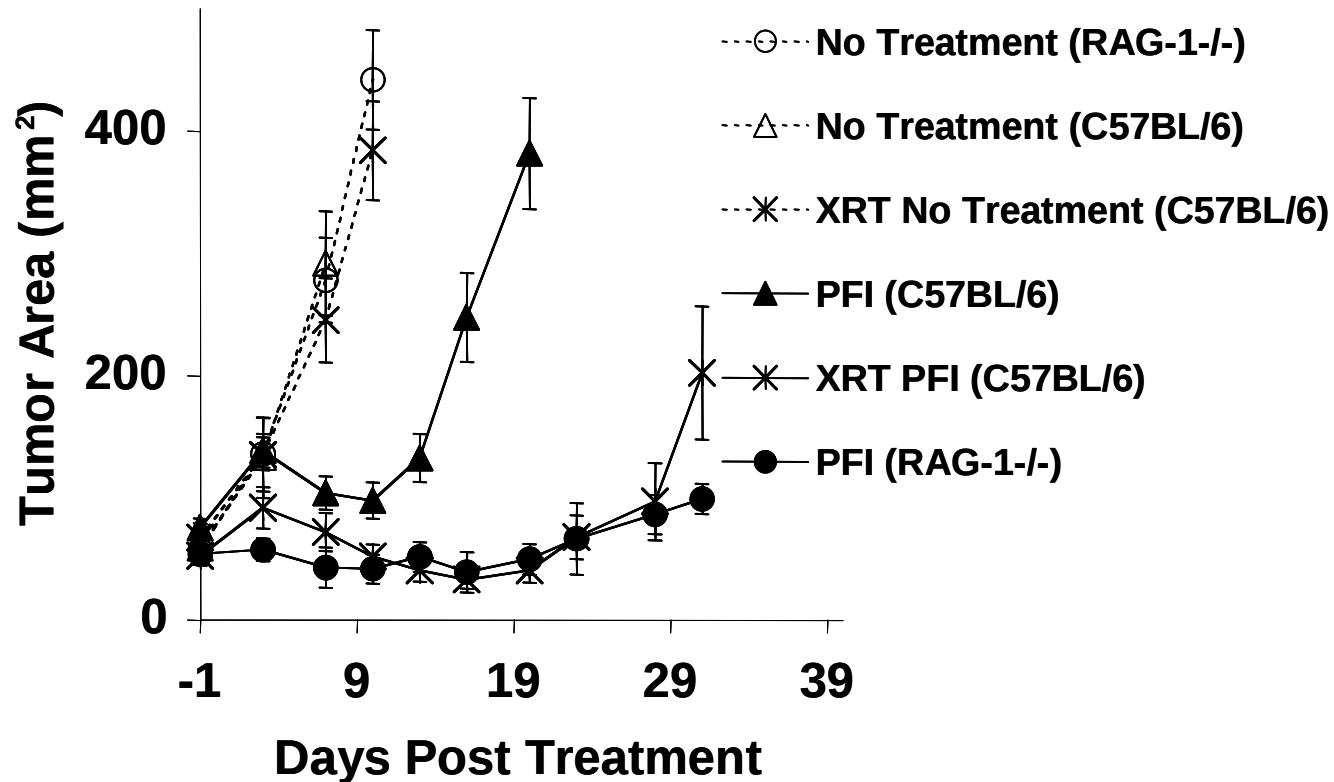
*Gattinoni et al. J exp med, 2005*



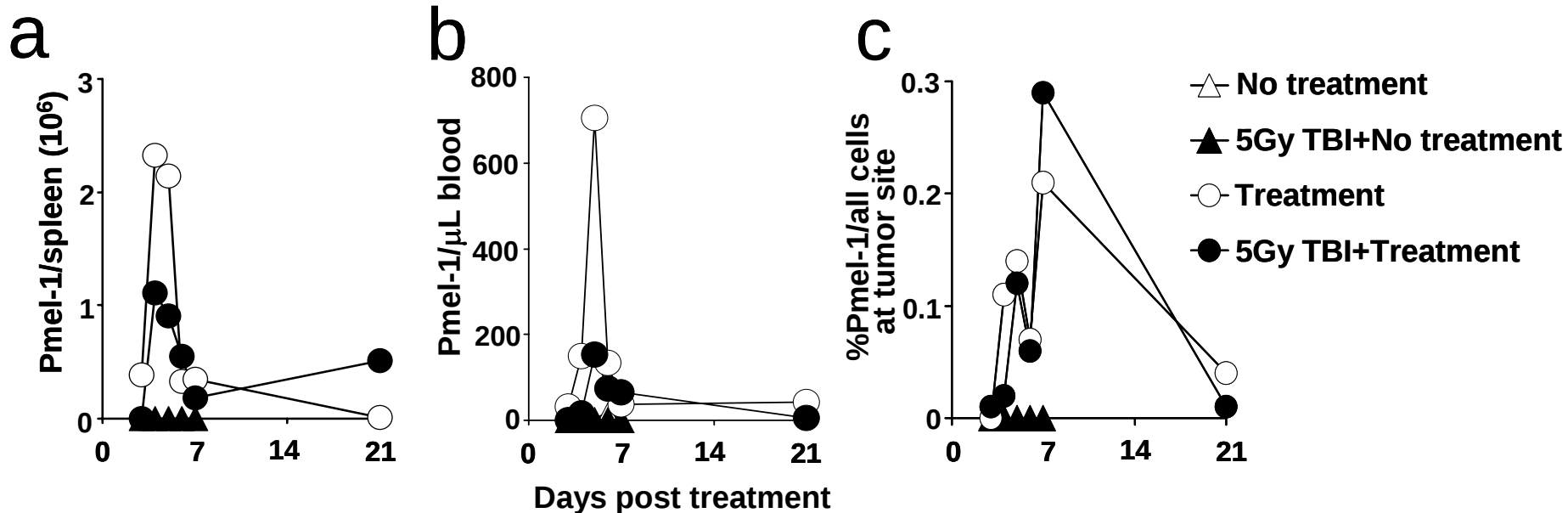
# Sublethal irradiation acts *via* indirect mechanisms rather than direct tumor killing



# Lymphodepletion by genetic means recapitulates the effect of sublethal irradiation



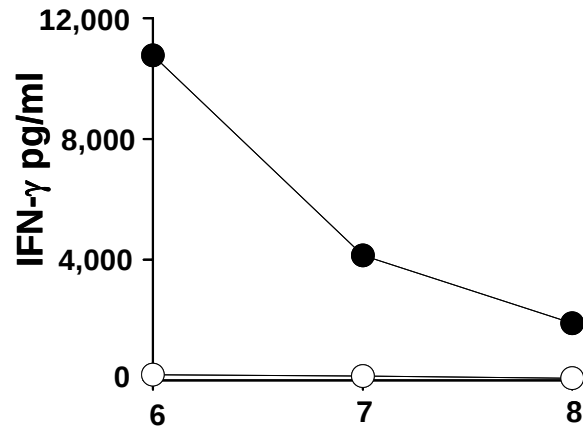
# Lymphodepletion does not result in increased numbers of transferred CD8<sup>+</sup> T cells



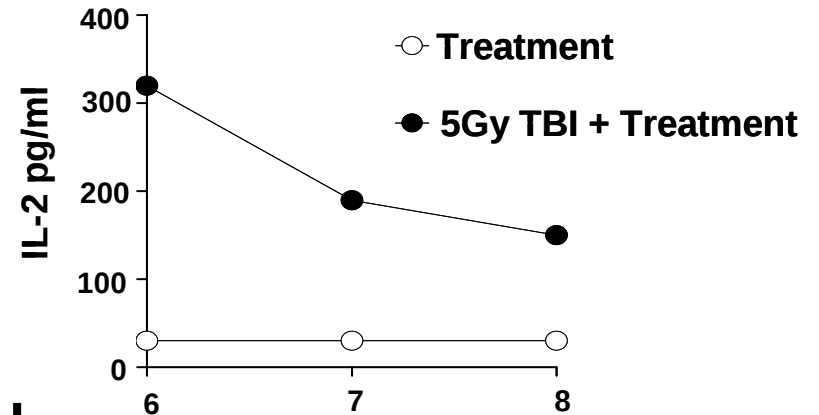
*Gattinoni et al. J exp med, 2005*

# Lymphodepletion augments the effector functions of transferred CD8<sup>+</sup> T cells

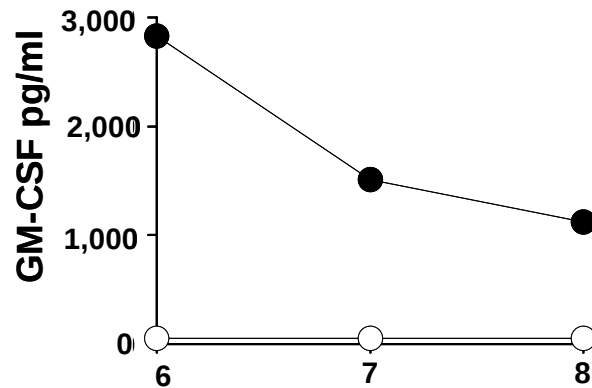
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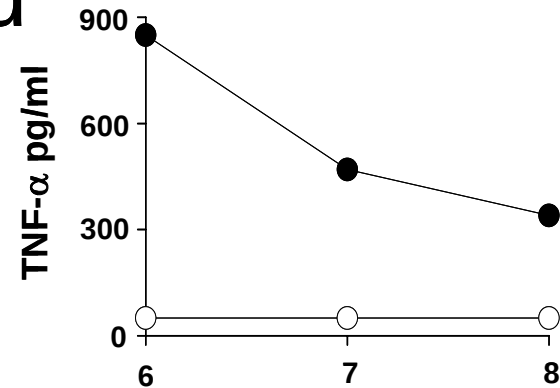
b



c

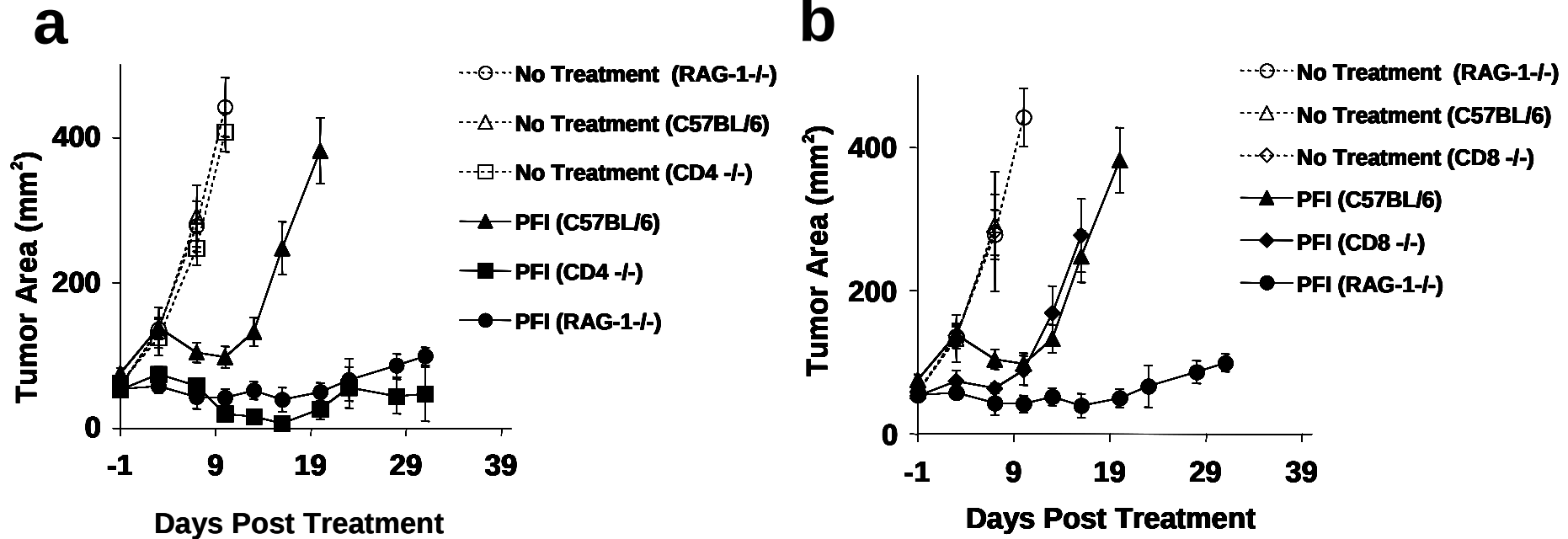


d



-log [hgp100<sub>25-33</sub>] (M)

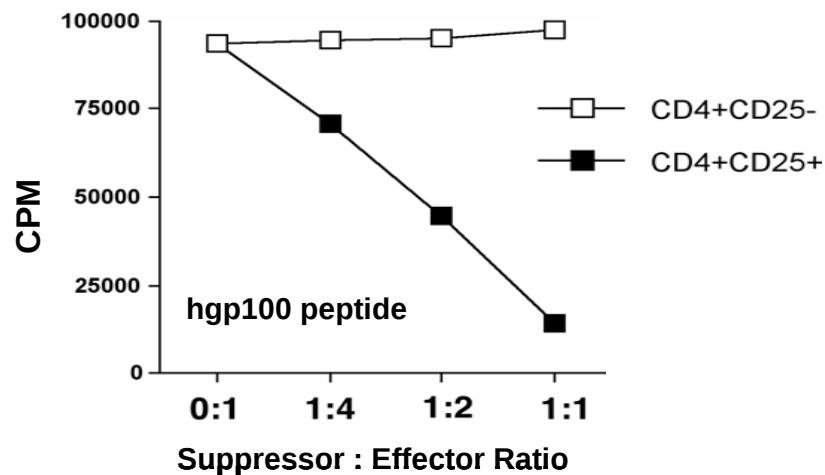
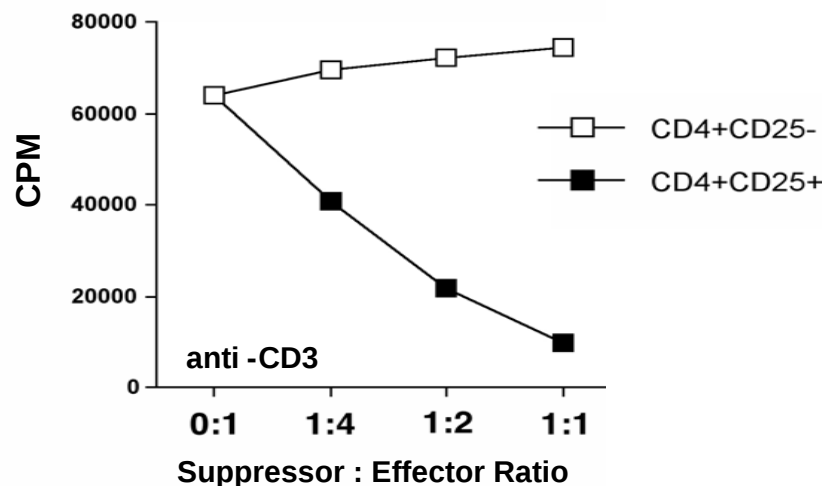
# Endogenous CD4<sup>+</sup> but not CD8<sup>+</sup> T cells suppress the anti-tumor activity of transferred CD8<sup>+</sup> T cells



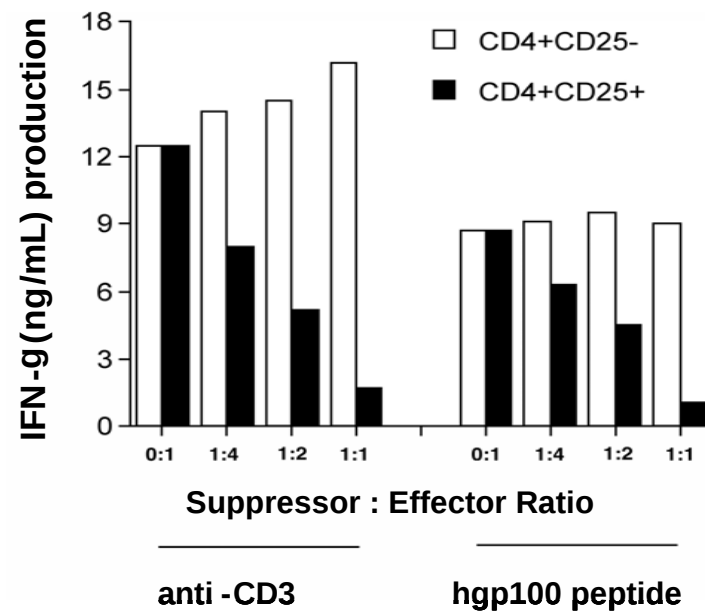
*Antony et al. JI, 2005*

# CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells suppress pmel-1 CD8<sup>+</sup>T cells *in vitro*

**a**

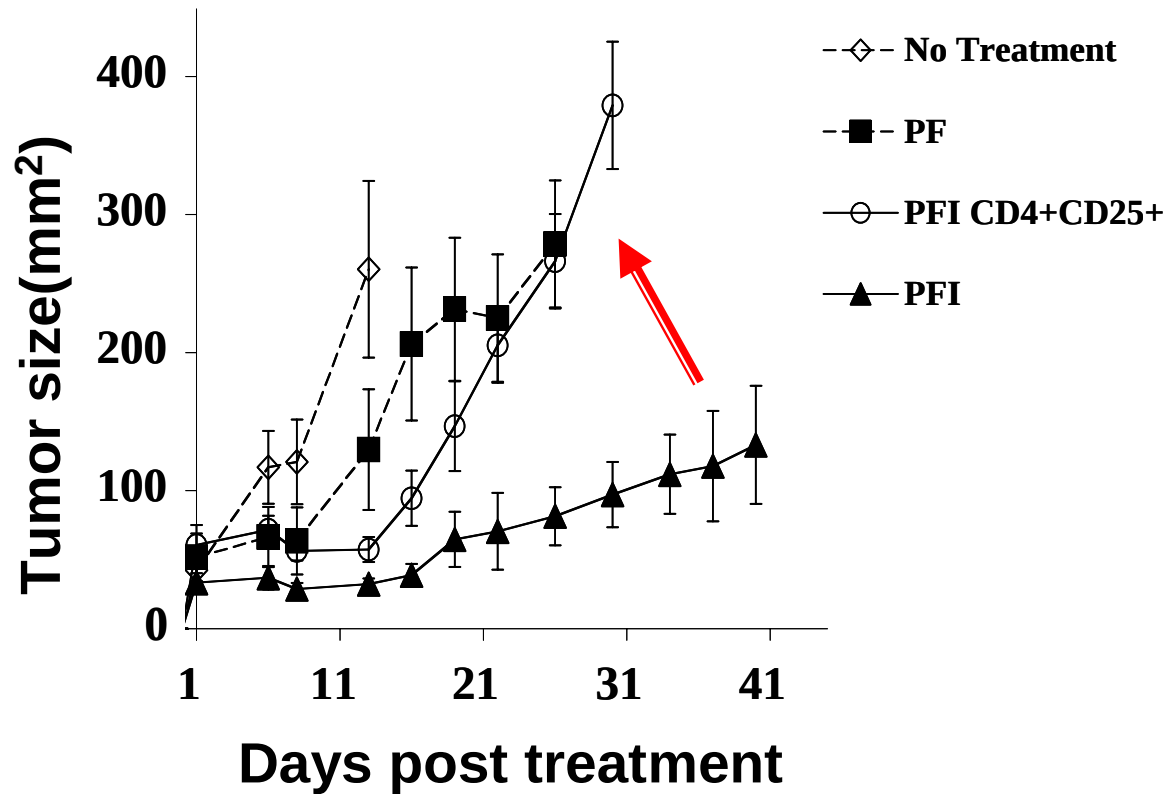


**b**

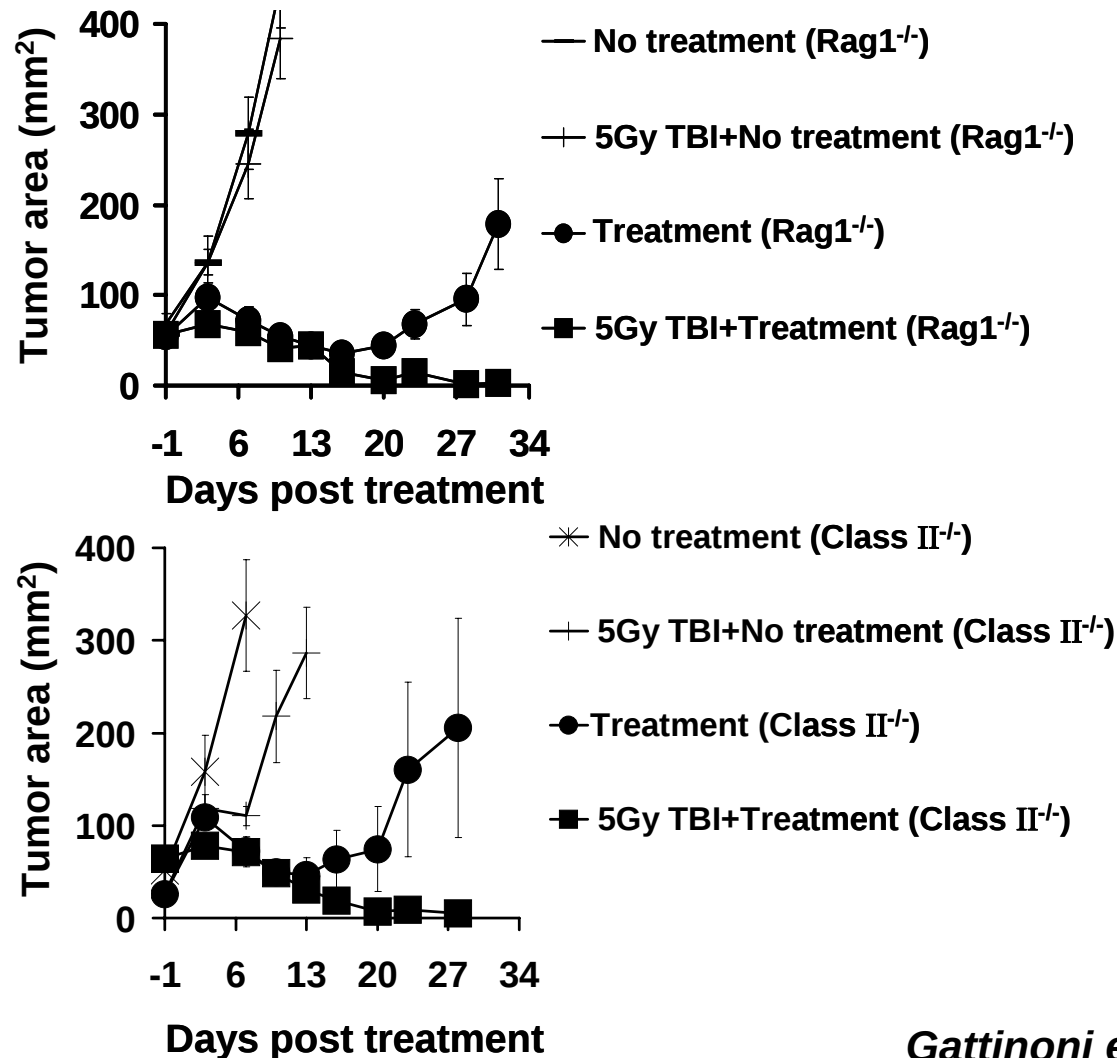


*Antony et al. JI, 2005*

# CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells suppress pmel-1 CD8<sup>+</sup>T cells *in vivo*

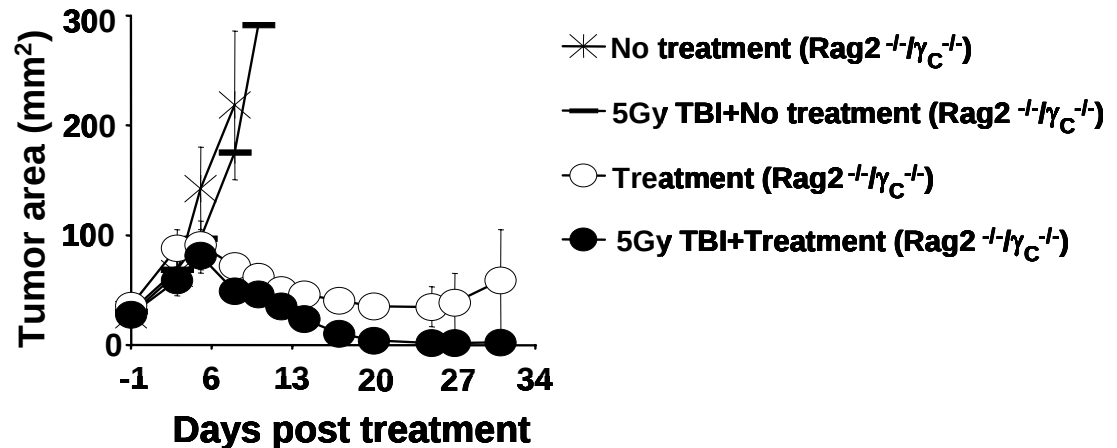
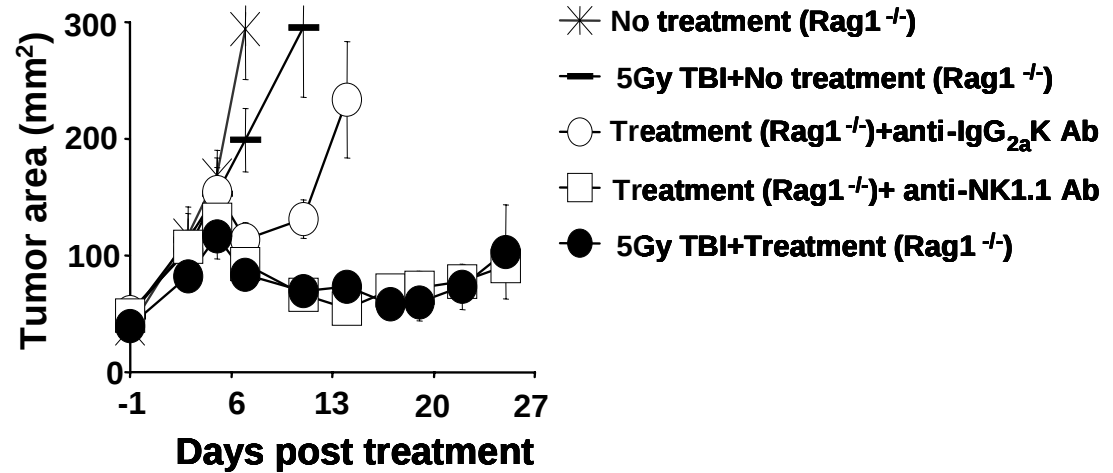
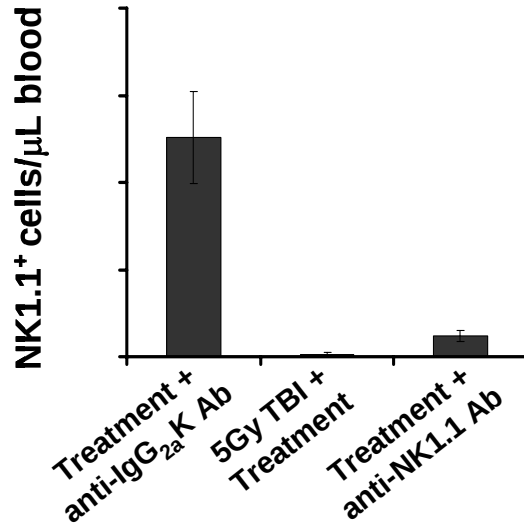


# Sublethal irradiation enhances the anti-tumor efficacy of transferred CD8<sup>+</sup> T cells even in the genetic absence of Tregs

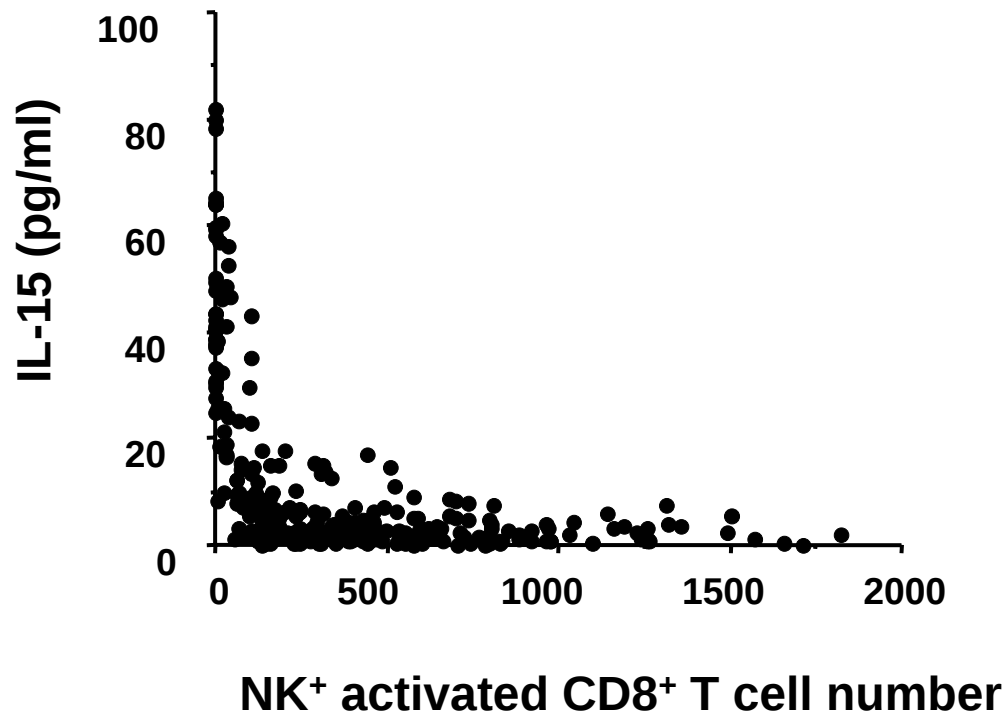


Gattinoni et al. J exp med, 2005

# Removal of NK cells enhances the anti-tumor efficacy of transferred CD8<sup>+</sup> T cells



# IL-15 levels correlate inversely with NK and activated CD8<sup>+</sup> T cell populations

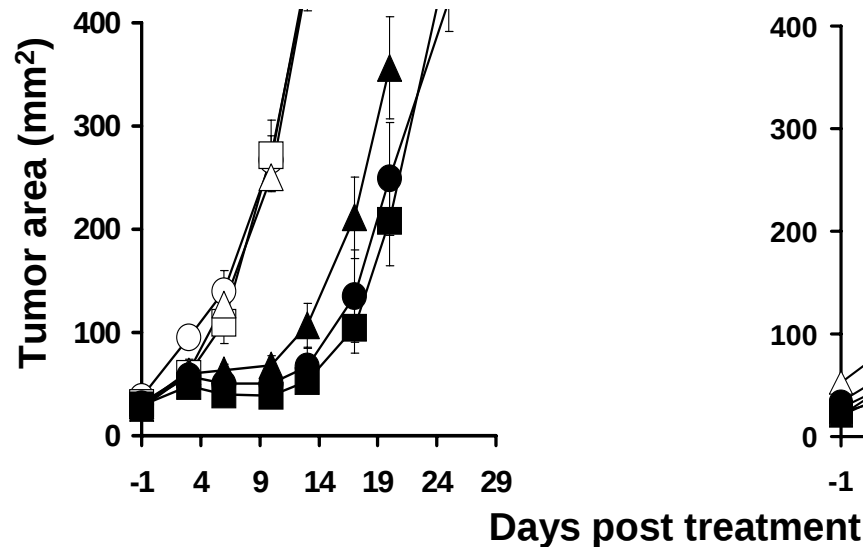


Gress RE, unpublished data

# Increased access to endogenous IL-15 and IL-7 into irradiated hosts enhances the anti-tumor efficacy of transferred CD8<sup>+</sup> T cells

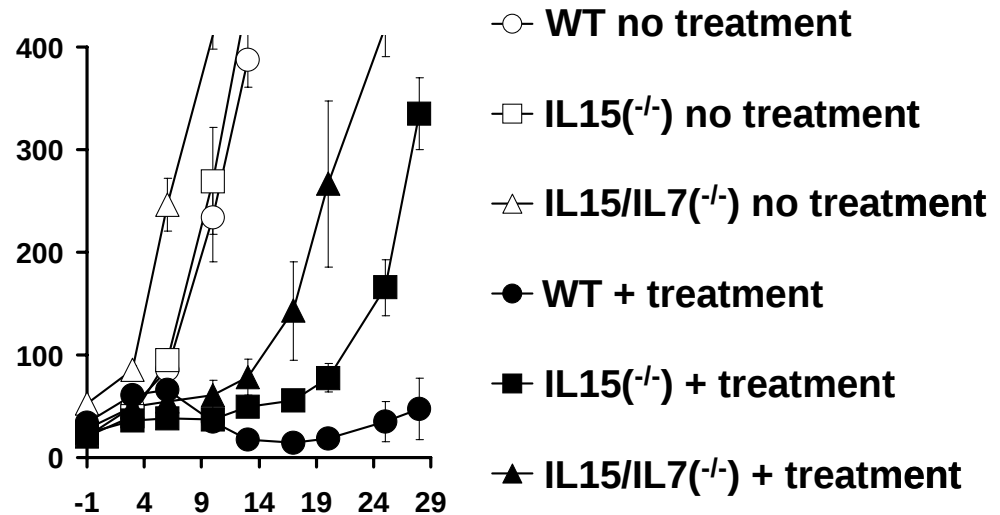
a

Non-irradiated host



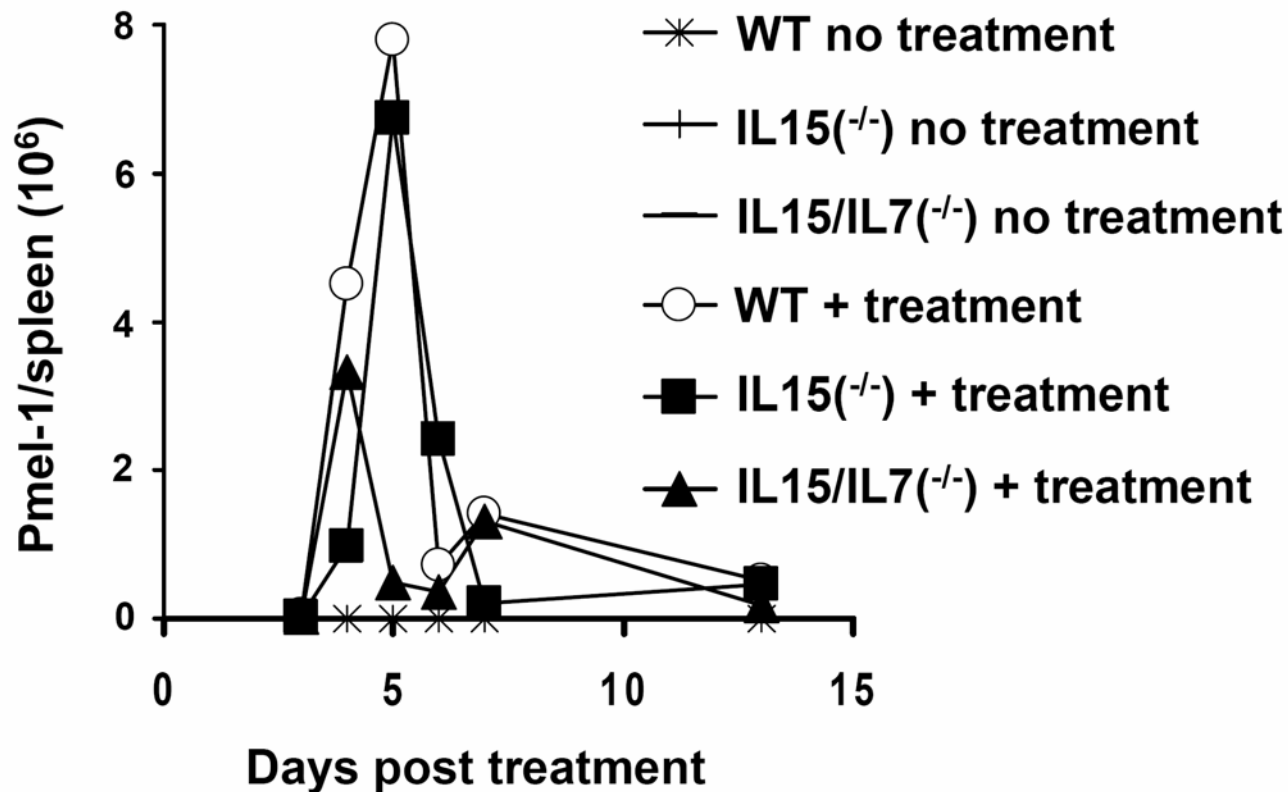
b

Irradiated host

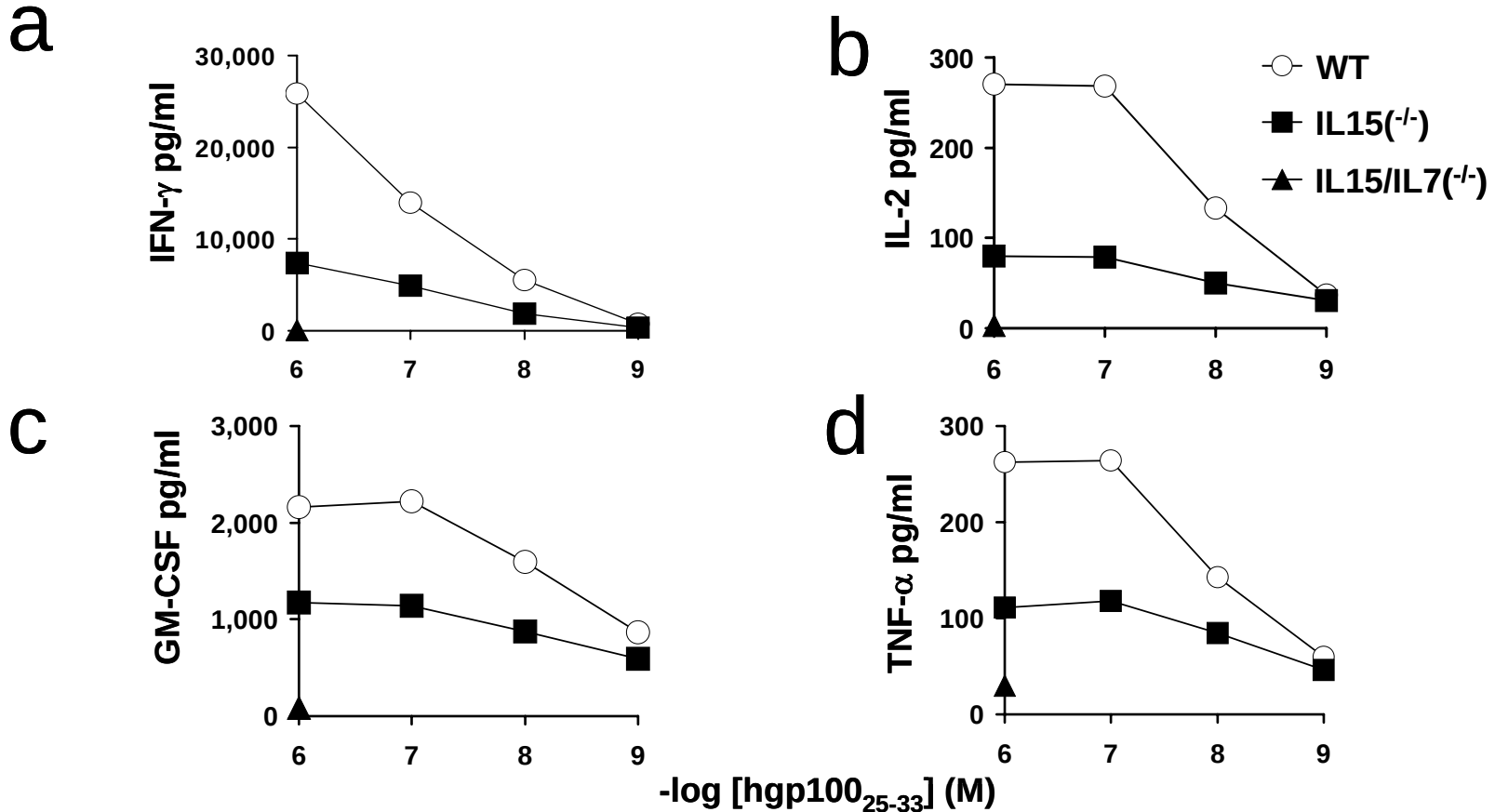


*Gattinoni et al. J exp med, 2005*

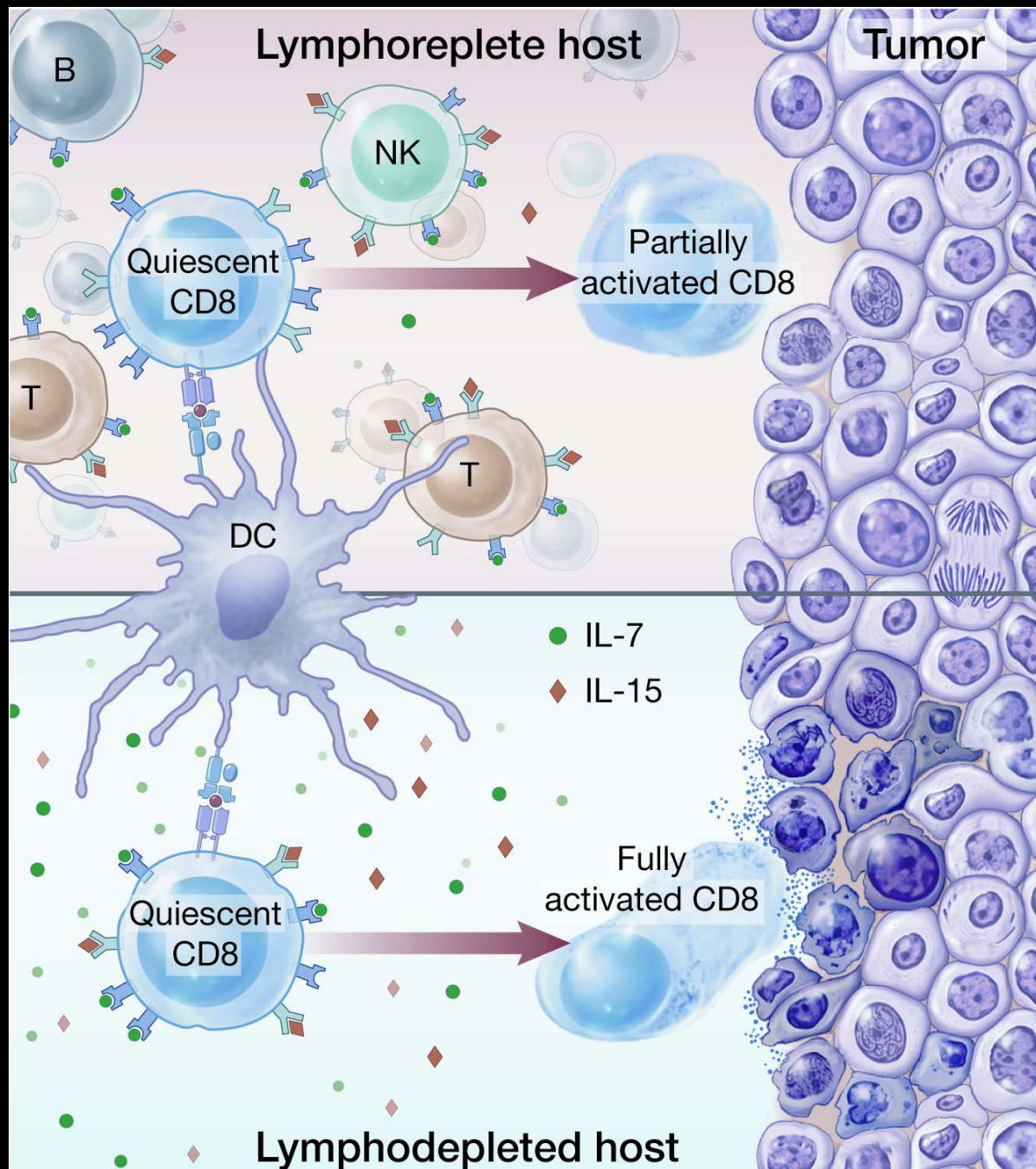
## Proliferative responses of transferred CD8<sup>+</sup> T cells are impaired in the absence of both IL-7 and IL-15



# Increased access to endogenous IL-15 and IL-7 enhances the effector functions of transferred CD8<sup>+</sup> T cells

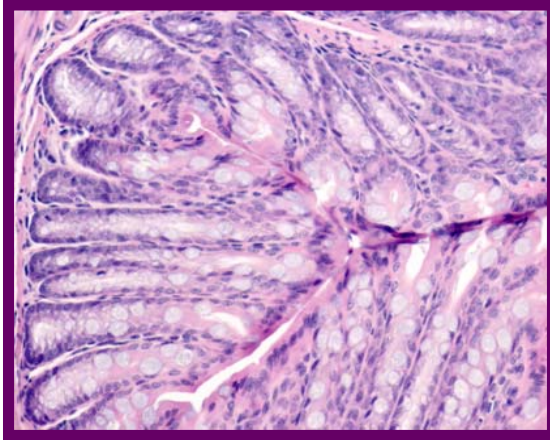


*Gattinoni et al. J exp med, 2005*

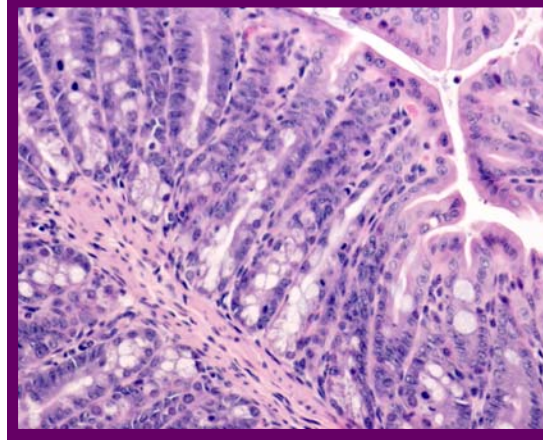


# Damage of the integrity of mucosal barriers by irradiation facilitate translocation of LPS into the blood stream

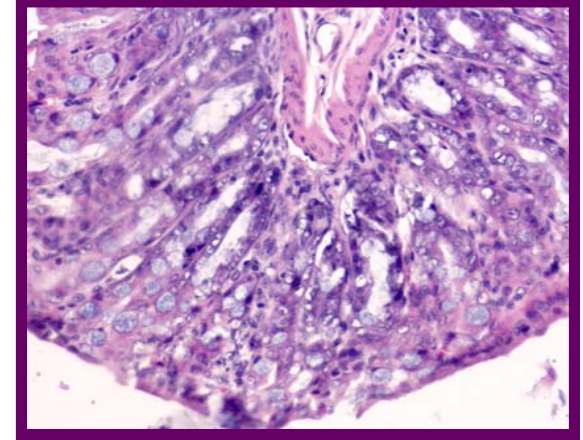
0 Gy



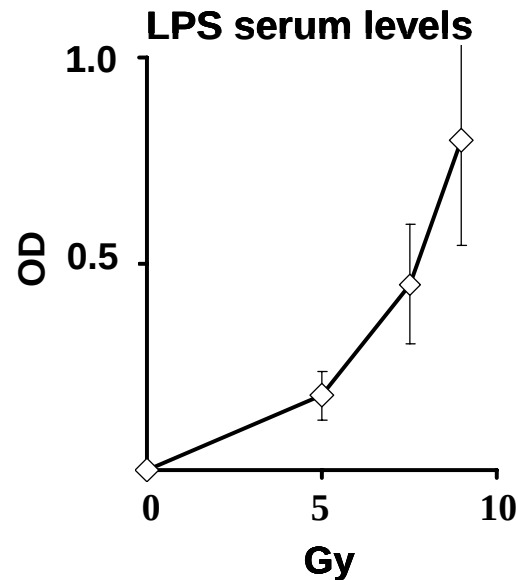
5 Gy



9 Gy

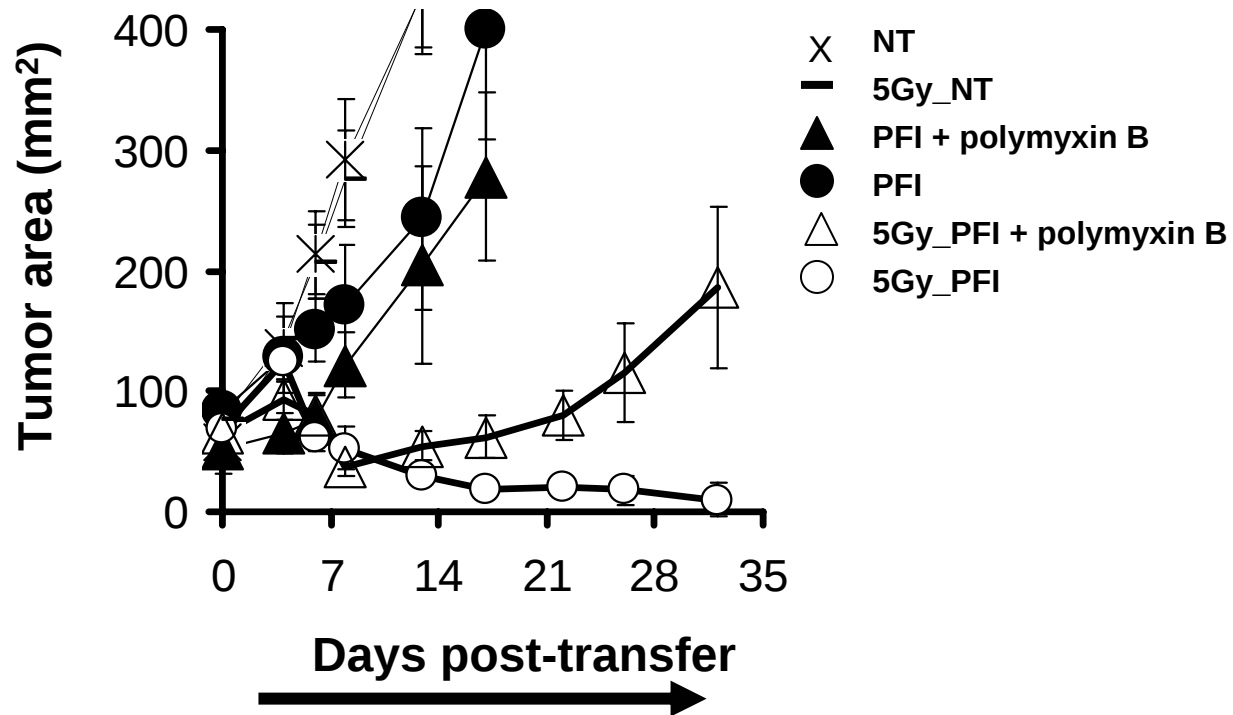


| Gy | Score   |
|----|---------|
| 0  | 0, 0, 0 |
| 5  | 1, 1, 2 |
| 9  | 2, 3, 3 |



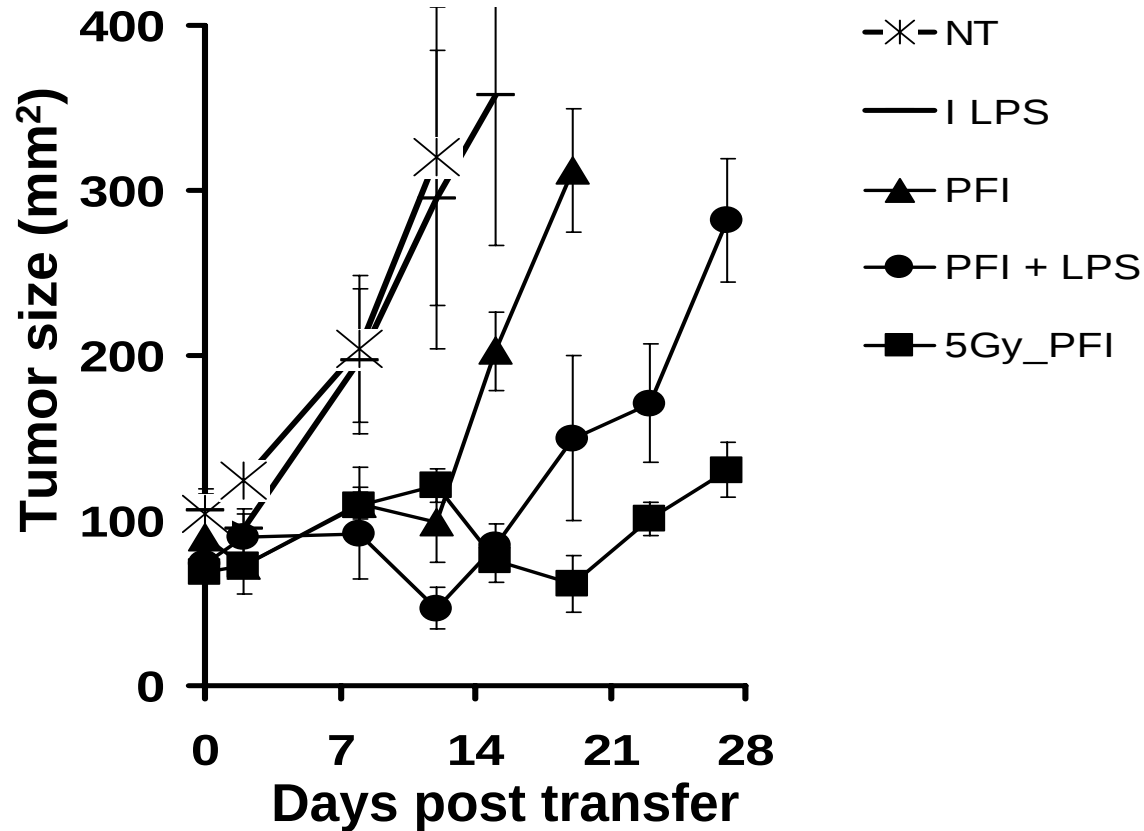
*Paulos et al. MS in preparation*

# Block of LPS by *Polymyxin B* partially impairs the effect of sublethal irradiation.



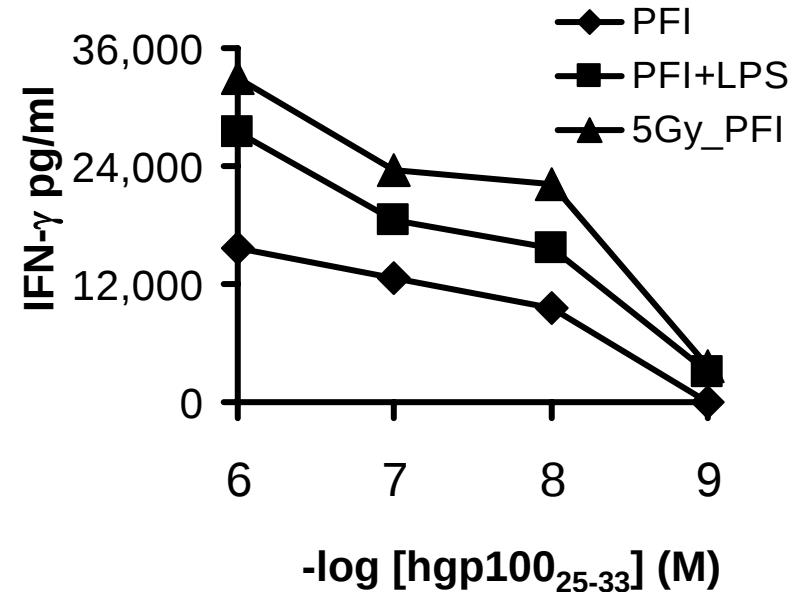
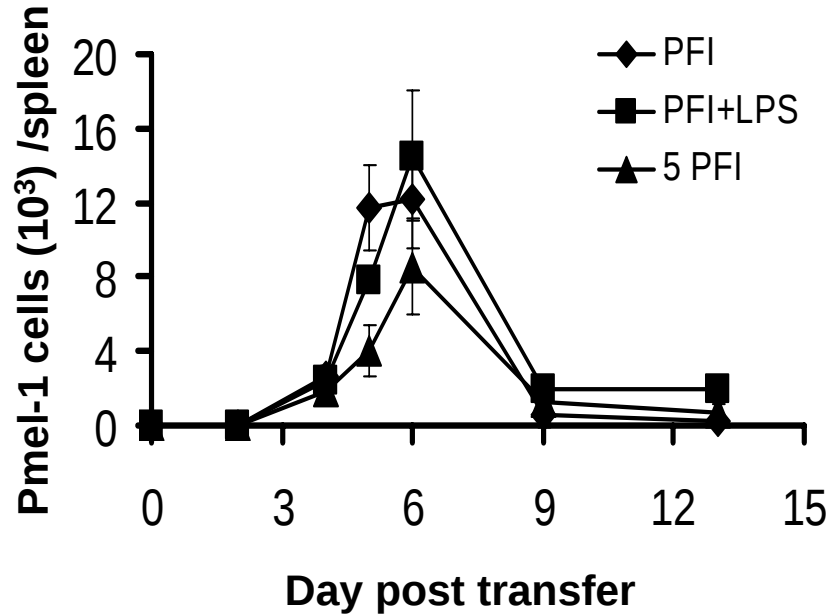
Continuous administration of  
Polymyxin B (10 µg/day for three weeks)

# Exogenous administration of LPS augments the anti-tumor efficacy of transferred CD8<sup>+</sup> T cells

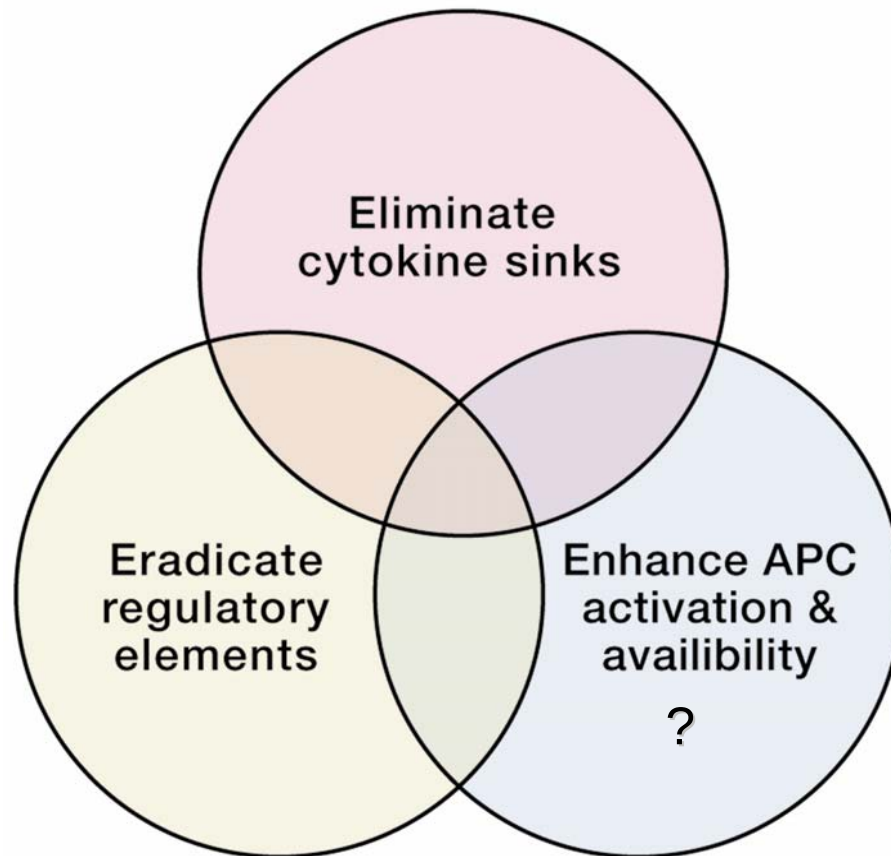


*Paulos et al. MS in preparation*

# LPS induces qualitative rather than quantitative improvement on transferred CD8<sup>+</sup> T cells



# An interactive model for the host mechanisms underlying the impact of lymphodepletion on adoptively-transferred-T cells

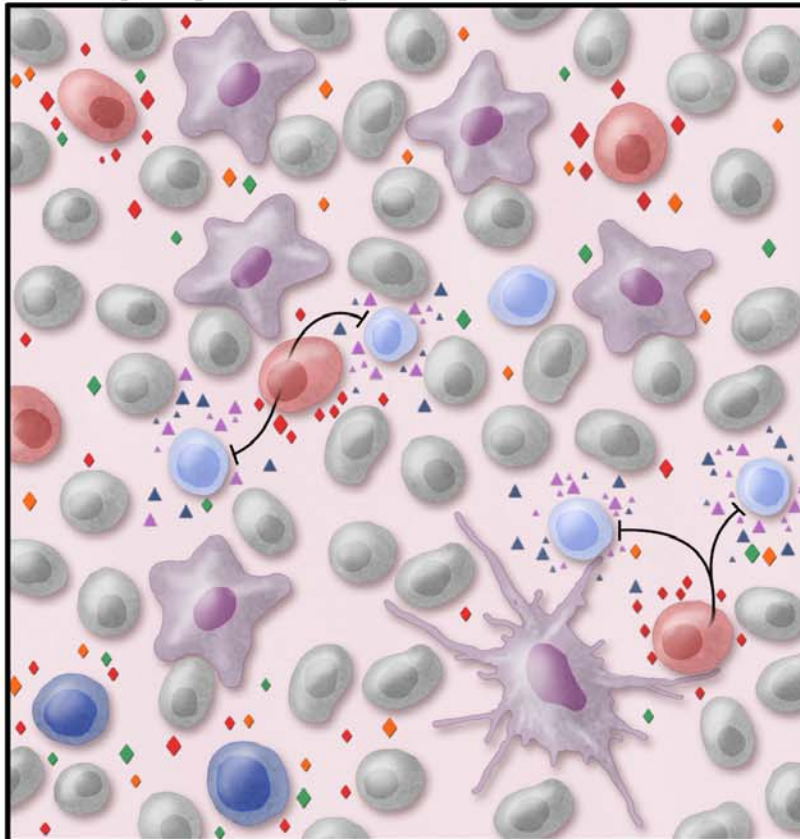


Wrzesinski, Current Opinion In Immunology, 2005

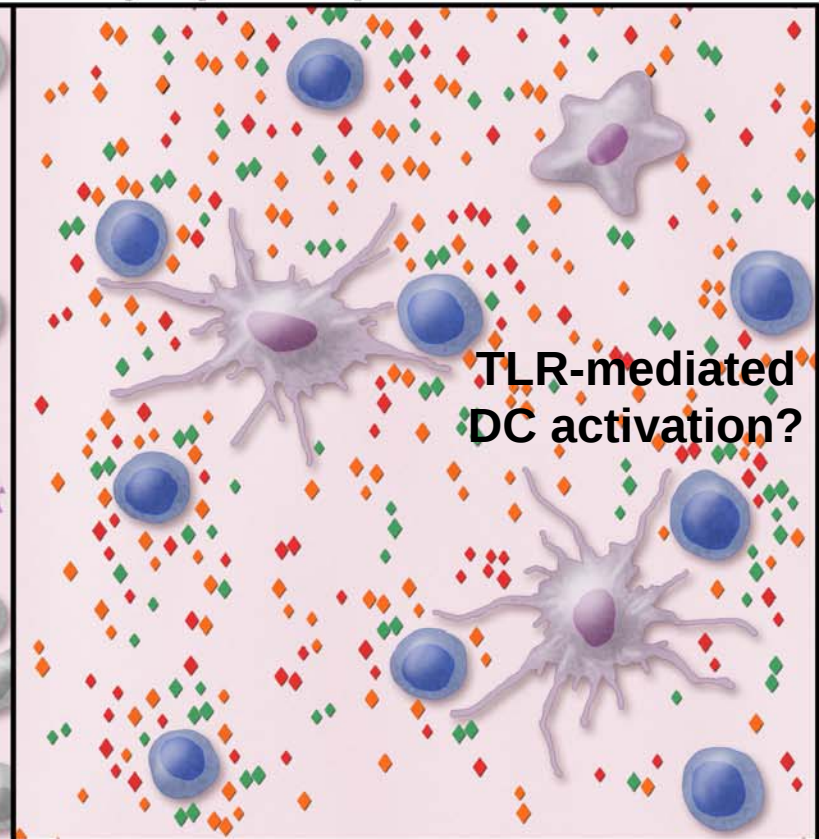
Klebanoff, Trends in Immunology, 2005

Paulos, MS in Preparation

## A. Lymphoreplete host



## B. Lymphodepleted host



TLR-mediated  
DC activation?

Competing lymphocyte

CTL

Fully activated CTL

Treg

TGF- $\beta$

IL-10

IL-2

IL-7

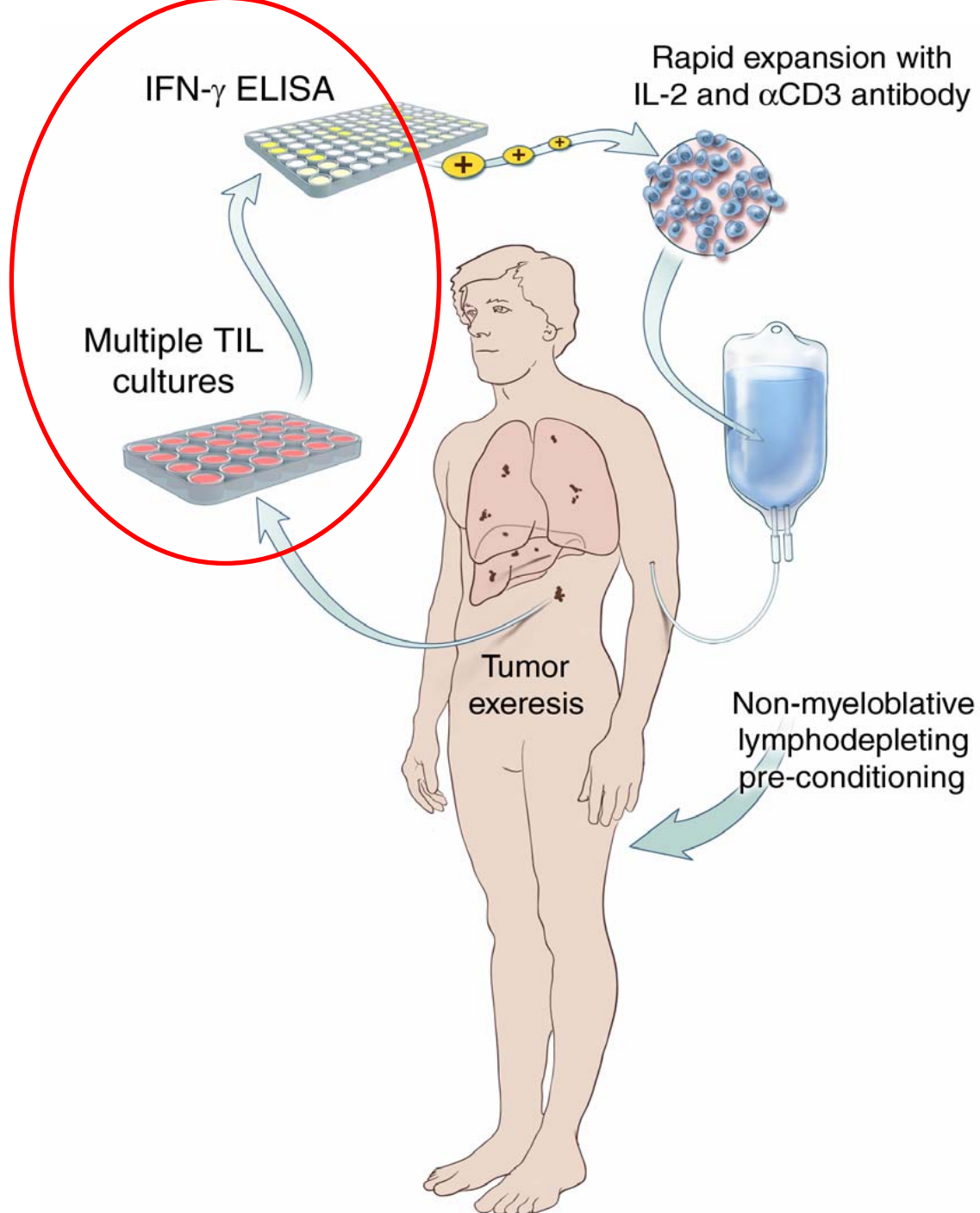
IL-15

Immature DC

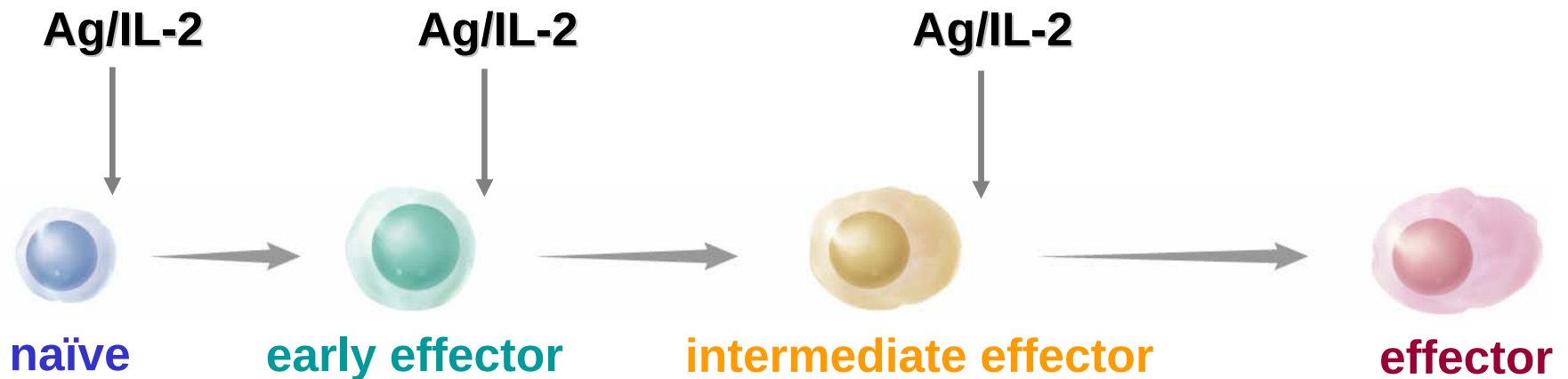
Mature DC

Wrzesinski, Current Opinion In Immunology, 2005  
Klebanoff, Trends in Immunology, 2005

Antony, J Immunol, 2005  
Paulos, MS in Preparation

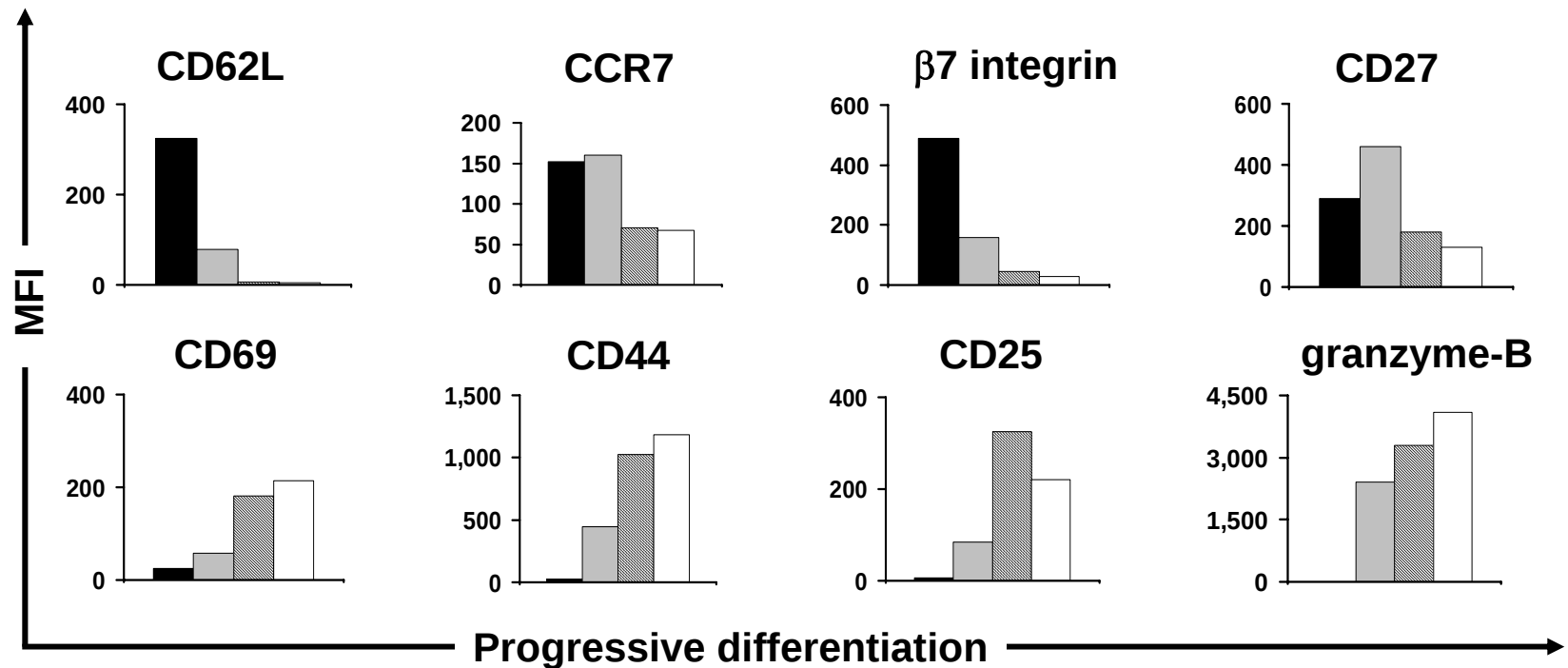


# *In vitro* generation of pmel-1 CD8<sup>+</sup> T cells at different stages of differentiation



# T cells differentiation stage was validated by FACS.....

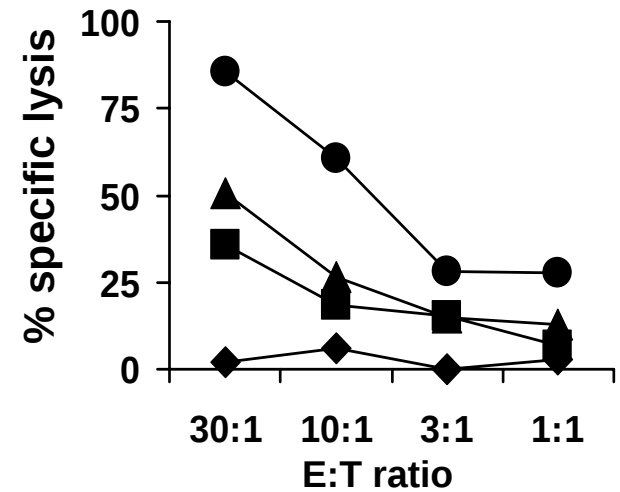
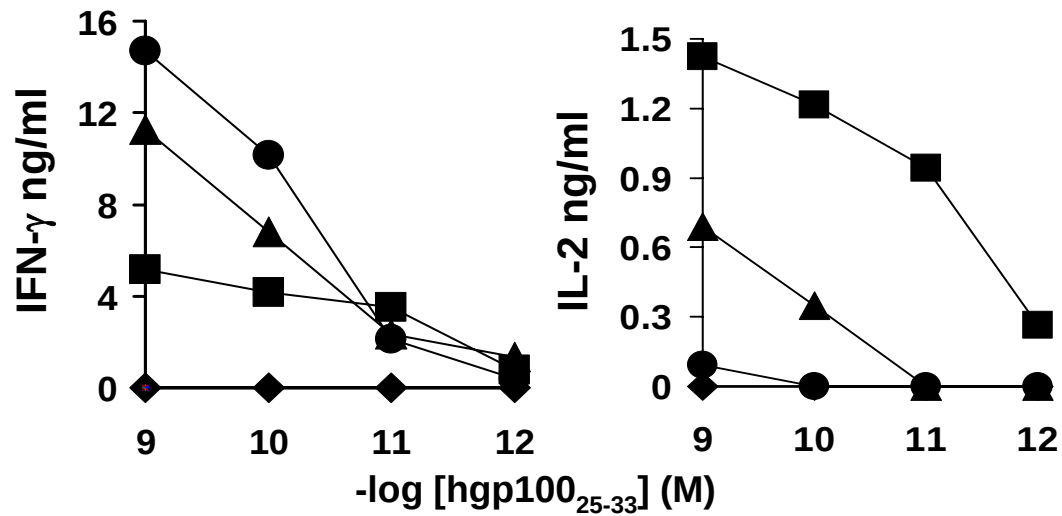
■ naive  
■ early effector  
▨ intermediate effector  
□ effector



*Gattinoni et al. JCI, 2005*

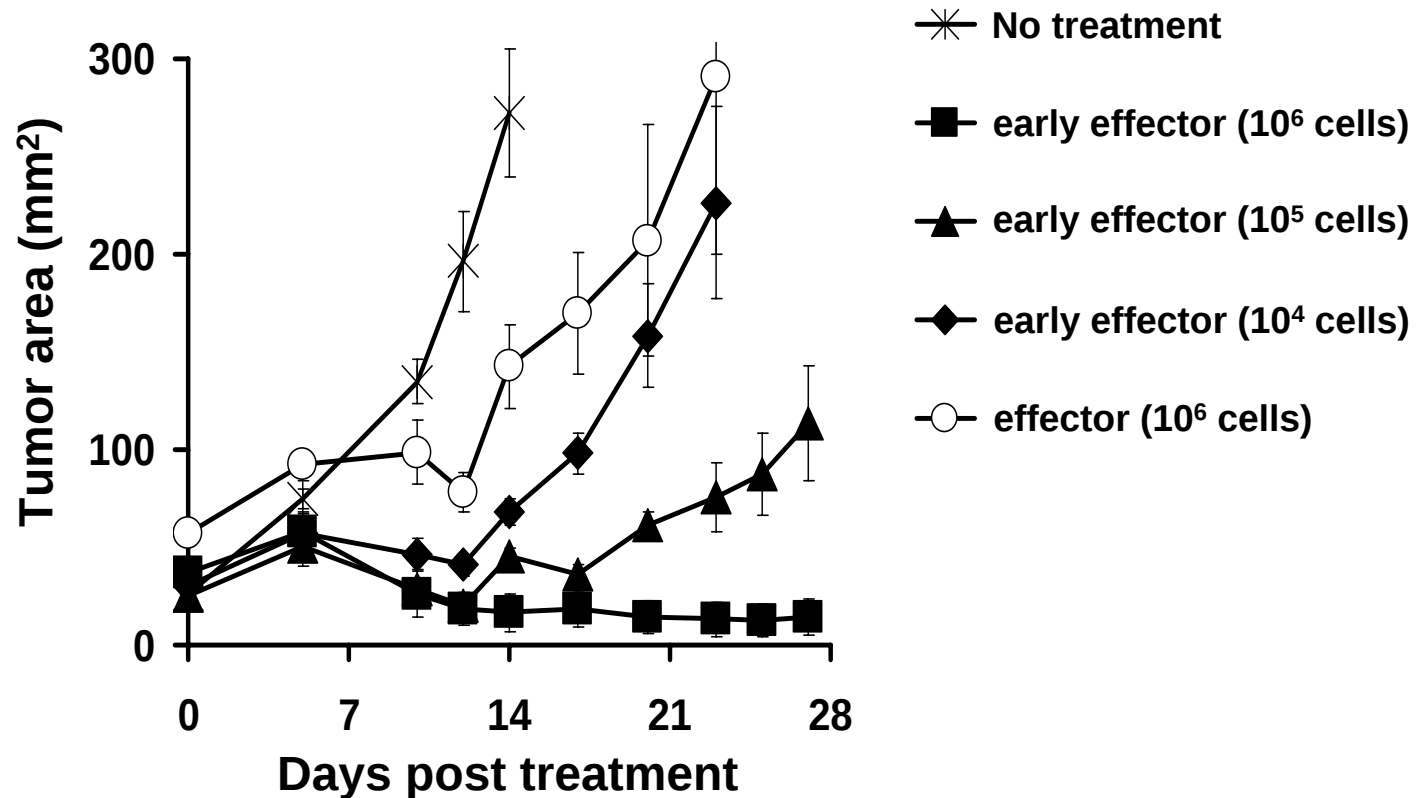
## ..... and functional analyses

◆ naïve      ▲ intermediate effector  
■ early effector      ● effector



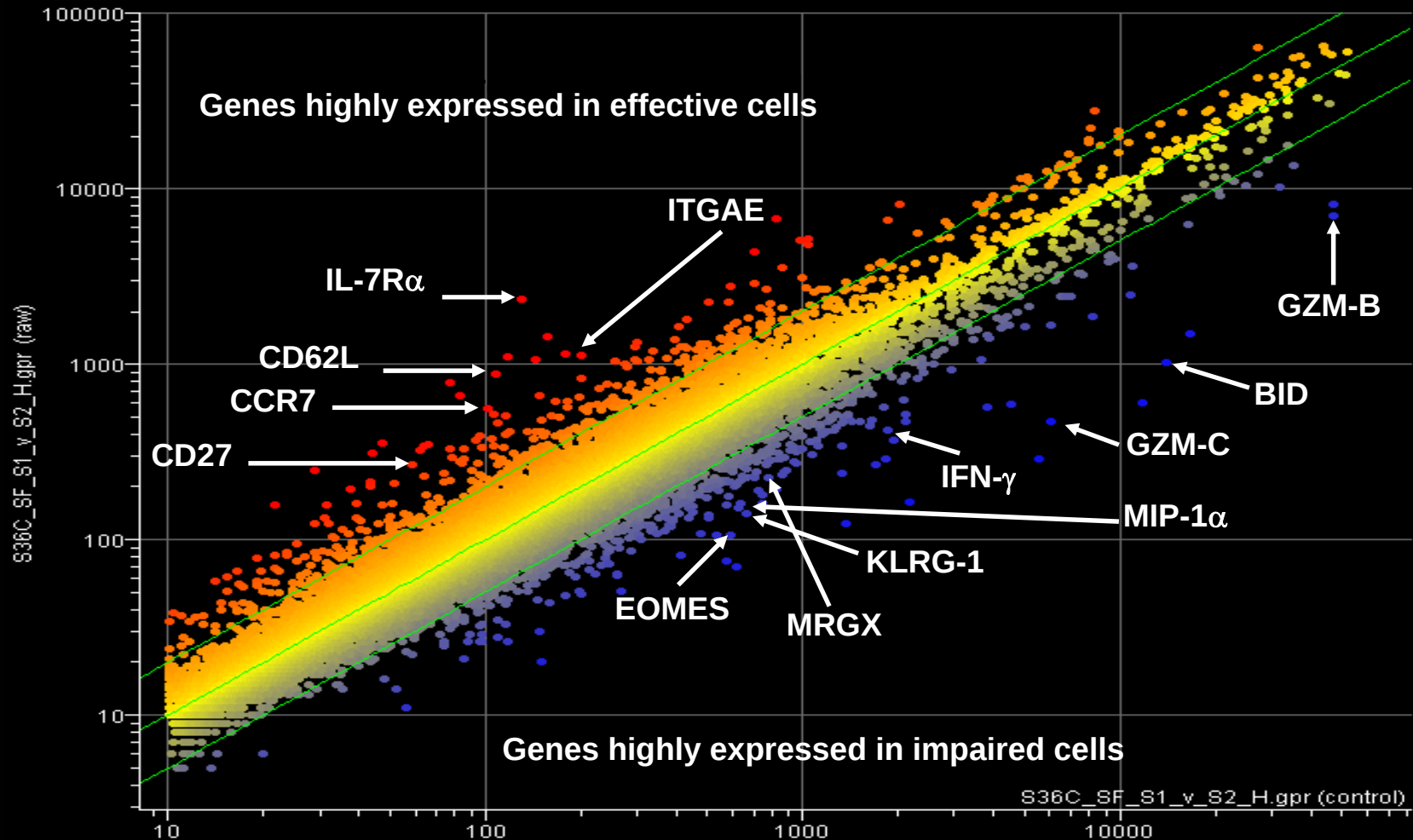
*Gattinoni et al. JCI, 2005*

# Acquisition of terminal effector function *in vitro* impairs *in vivo* anti-tumor efficacy



Gattinoni et al. JCI, 2005

# Further characterization of effective and impaired T cells



X-axis: S36C\_SF\_S1\_v\_2 (All Samples) : S36C\_SF\_S1\_v\_2 (All Samples)  
 Y-axis: S36C\_SF\_S1\_v\_2 (All Samples) : S36C\_SF\_S1\_v\_2 (All Samples)

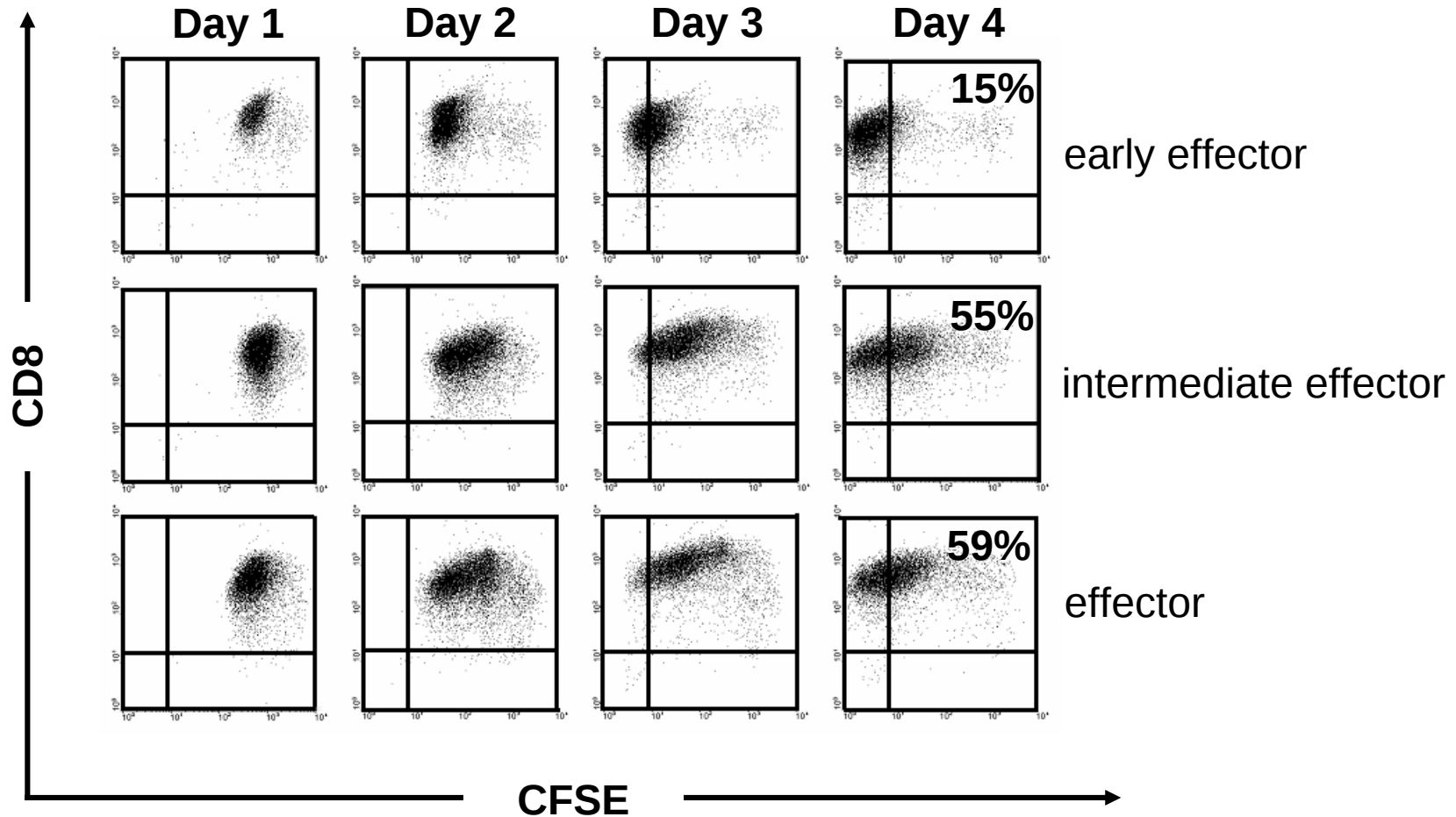
Colored by: S36C\_SF\_S1\_v\_2, All Samples (S36C\_SF\_S1\_v\_2, All Samples)  
 Gene List: all genes (21921), RIKEN cDNA 4930529I22 ...

| Genes highly expressed in effective cells |                  | Genes highly expressed in impaired cells |                  |
|-------------------------------------------|------------------|------------------------------------------|------------------|
| Lymphoid-homing                           | E:I fold changes | Effector functions                       | I:E fold changes |
| CD62L                                     | 5.6              | Granzyme A                               | 5.1              |
| CCR7                                      | 3.7              | Granzyme B                               | 6.0              |
| Integrin $\alpha$ E                       | 3.1              | Granzyme C                               | 6.0              |
| Co-stimulatory molecules                  |                  | Granzyme D                               | 3.5              |
|                                           |                  | Granzyme E                               | 2.9              |
| T cell survival / memory generation       |                  | Granzyme F                               | 3.9              |
|                                           |                  | Granzyme G                               | 3.1              |
|                                           |                  | Granzyme K                               | 5.0              |
|                                           |                  | Perforin                                 | 2.4              |
|                                           |                  | FASL                                     | 2.4              |
| IL-7R- $\alpha$                           | 7.4              | IFN- $\gamma$                            | 3.4              |
| CD27                                      | 2.9              | Eomesodermin                             | 4.5              |
|                                           |                  | <b>Apoptosis</b>                         |                  |
|                                           |                  | BID                                      | 10.7             |
|                                           |                  | BAD                                      | 4.4              |
|                                           |                  | FASL                                     | 2.4              |
|                                           |                  | <b>Replicative senescence</b>            |                  |
|                                           |                  | KLRG-1                                   | 2.5              |
|                                           |                  | MRGX                                     | 2.3              |

E, effective cells (early effector)  
I, impaired cells (intermediate effector)

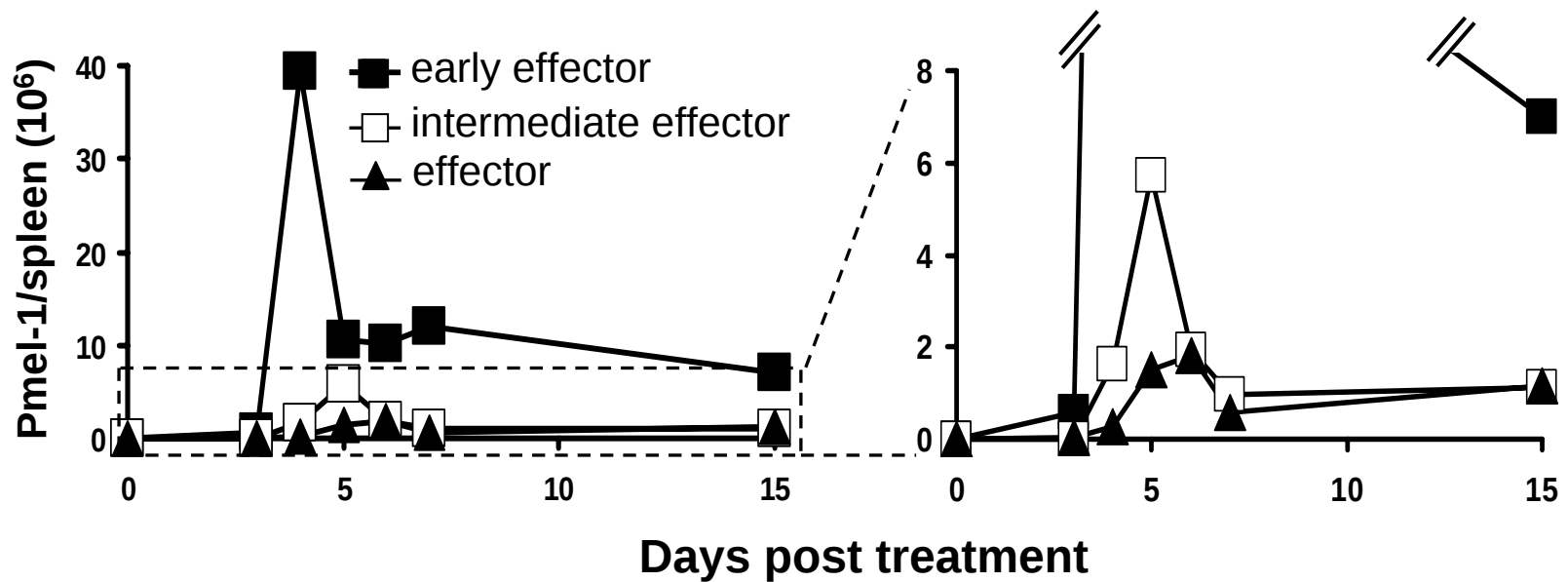
*Gattinoni et al. JCI, 2005*

# The differentiation state of CD8<sup>+</sup> T cells is inversely related to their proliferative capacity



*Gattinoni et al. JCI, 2005*

# The differentiation state of CD8<sup>+</sup> T cells is inversely related to their proliferative capacity



*Gattinoni et al. JCI, 2005*

## Naive



CD62L<sup>+++</sup>  
CCR7<sup>+++</sup>  
CD27<sup>+++</sup>  
CD28<sup>+++</sup>  
CD45RA<sup>+++</sup>  
CD45RO<sup>-</sup>  
KLRG-1<sup>-</sup>  
CD57<sup>-</sup>  
Telomere<sup>LONG</sup>

## Early Effector



CD62L<sup>++</sup>  
CCR7<sup>++</sup>  
CD27<sup>++</sup>  
CD28<sup>++</sup>  
CD45RA<sup>-</sup>  
CD45RO<sup>+++</sup>  
KLRG-1<sup>-</sup>  
CD57<sup>-</sup>  
Telomere<sup>LONG/INTERMEDIATE</sup>

## Intermediate Effector

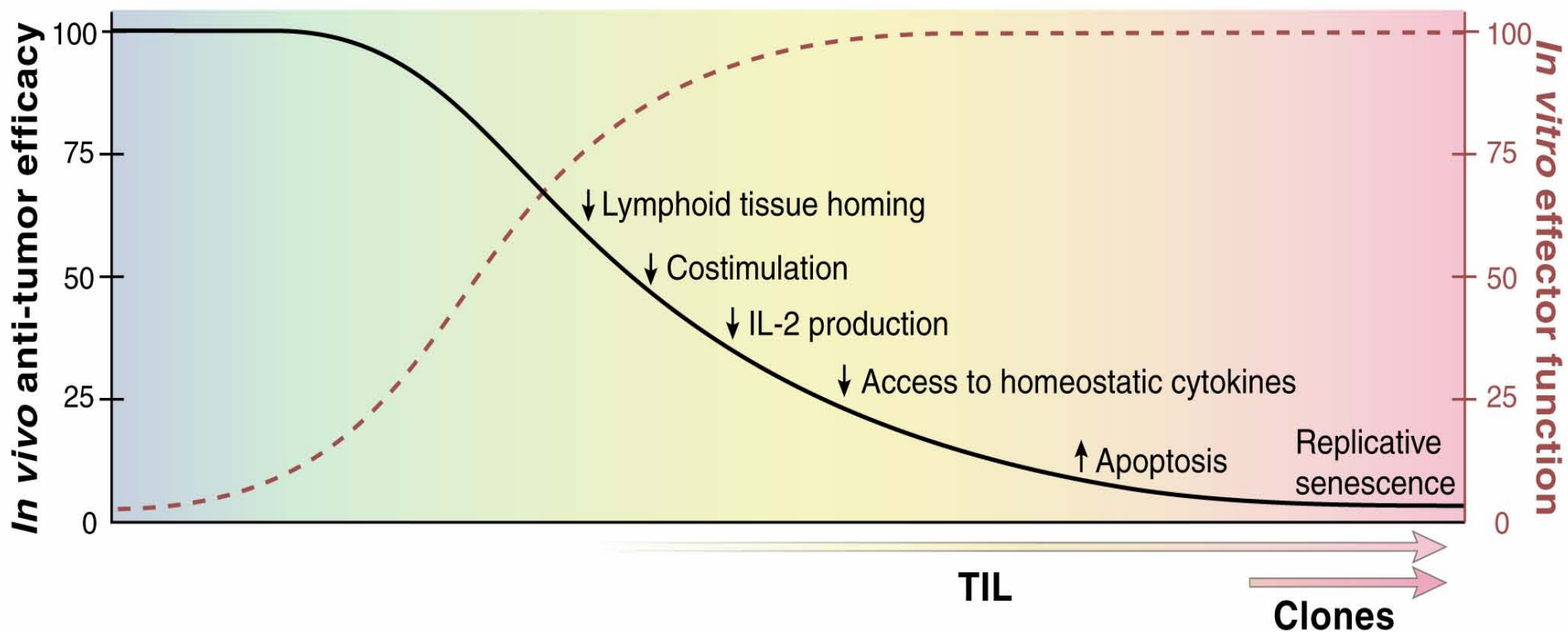


CD62L<sup>-</sup>  
CCR7<sup>-</sup>  
CD27<sup>++</sup>  
CD28<sup>-</sup>  
CD45RA<sup>-</sup>  
CD45RO<sup>+++</sup>  
KLRG-1<sup>+</sup>  
CD57<sup>+</sup>  
Telomere<sup>INTERMEDIATE</sup>

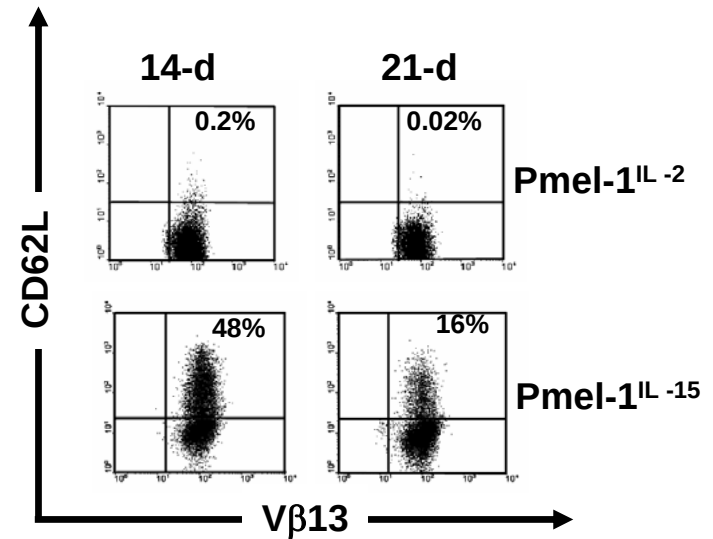
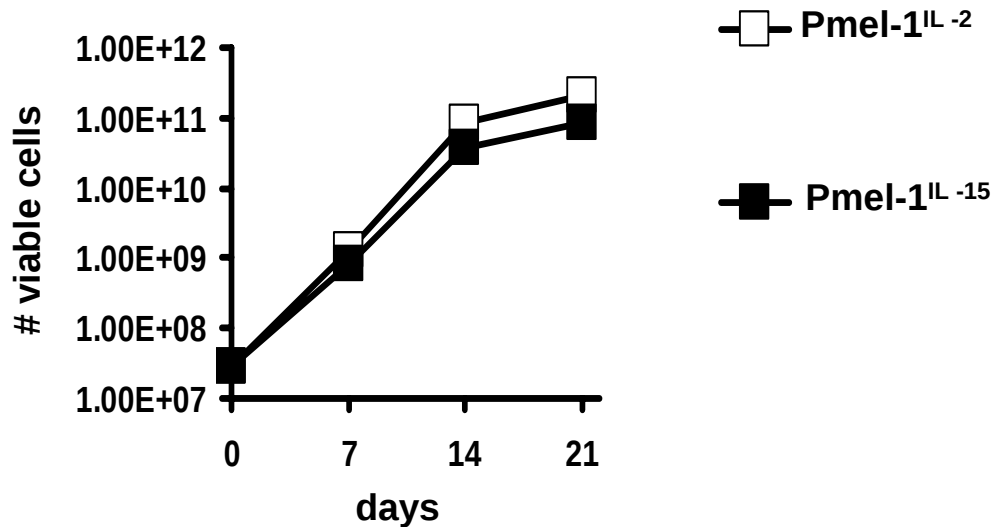
## Late Effector



CD62L<sup>-</sup>  
CCR7<sup>-</sup>  
CD27<sup>-</sup>  
CD28<sup>-</sup>  
CD45RA<sup>-/+</sup>  
CD45RO<sup>++</sup>  
KLRG-1<sup>+++</sup>  
CD57<sup>+++</sup>  
Telomere<sup>SHORT</sup>

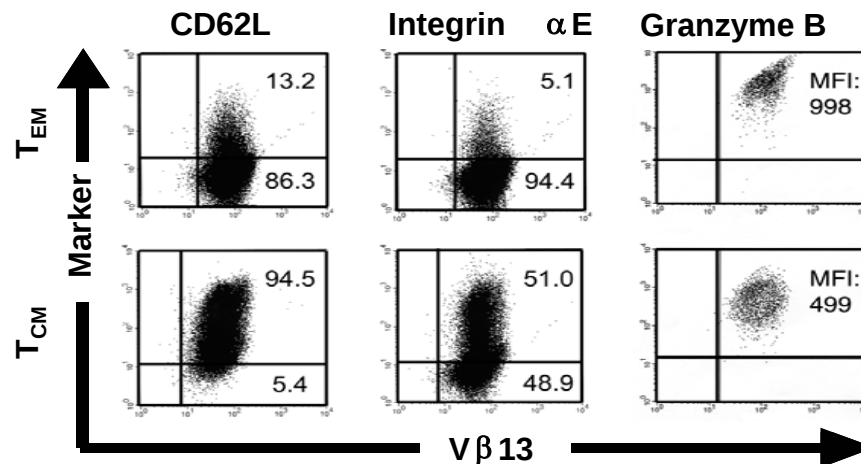
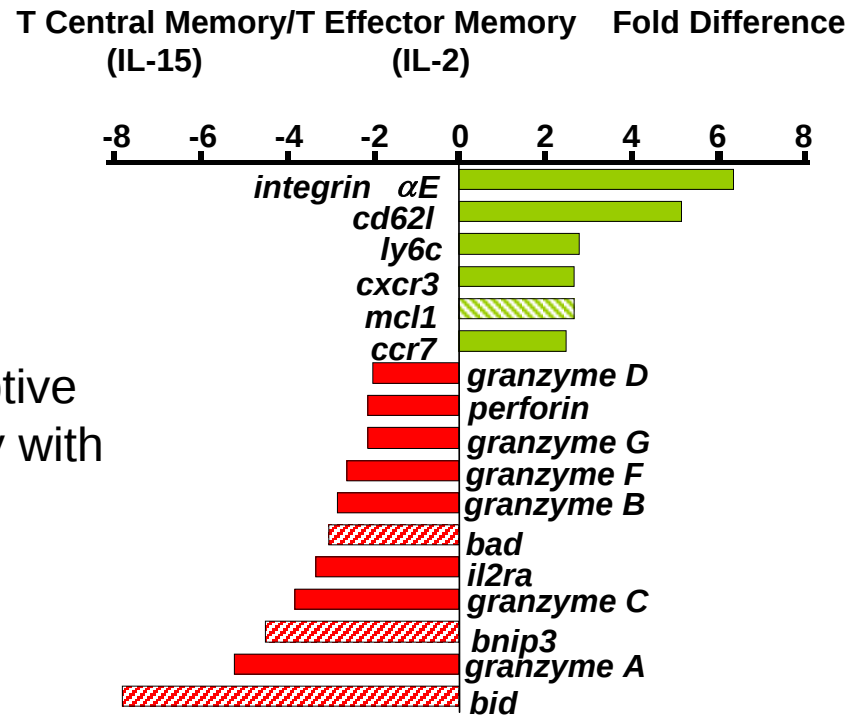


# Can we generate and select more effective CD8<sup>+</sup> T cells?



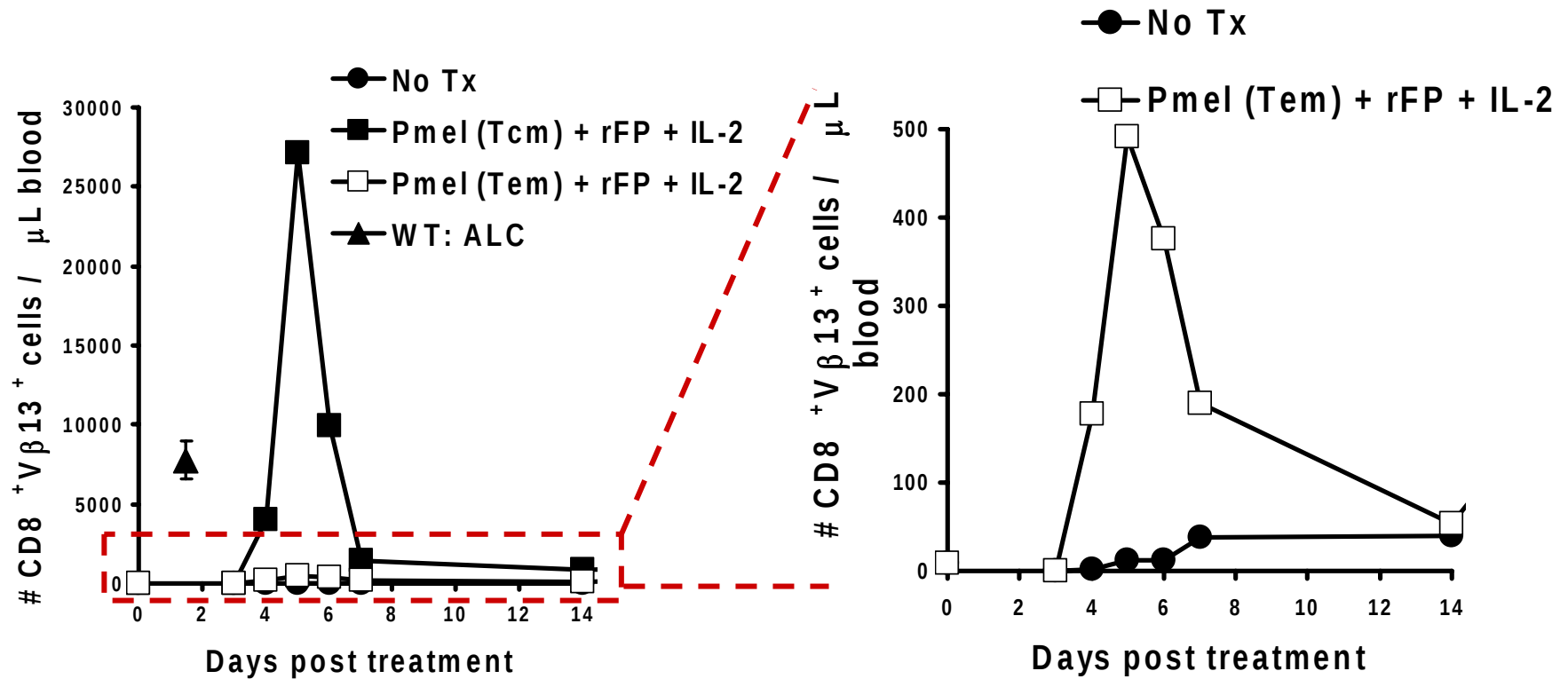
*Gattinoni et al. JCI, 2005*

Programming  
T cells for adoptive  
immunotherapy with  
IL-15



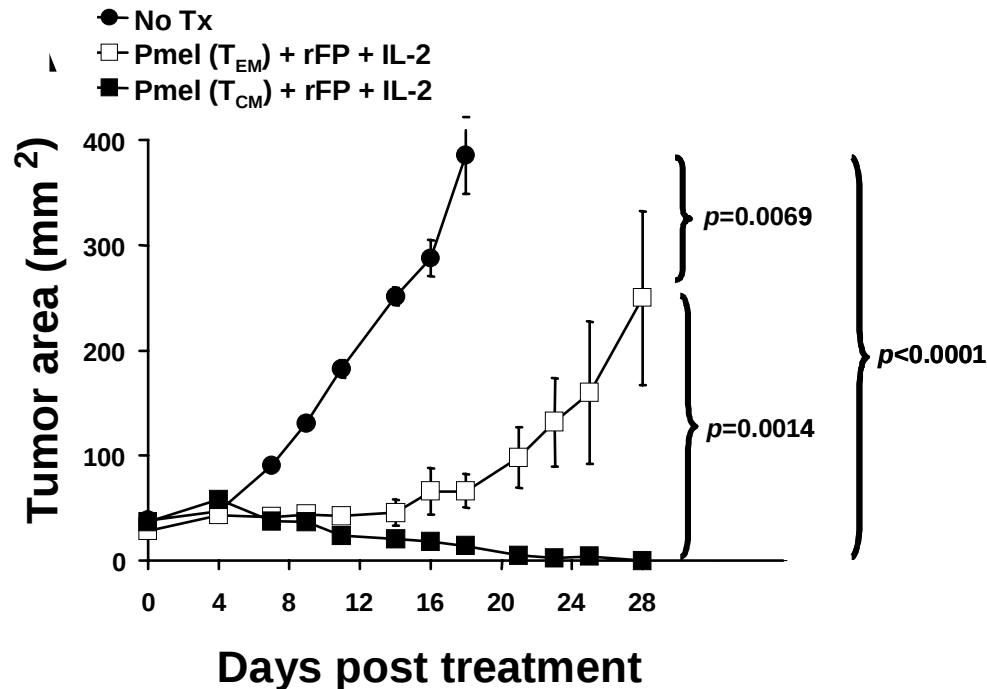
Klebanoff, et al, PNAS, 2005

# Explosive growth of central memory tumor-specific T cells



Klebanoff, et al, PNAS, 2005

# Programming T cells with IL-15 improves their efficacy in adoptive Immunotherapy



# Acknowledgements

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Pawel Muranski  
Lydie Cassard  
Andrea Boni  
Andrew Kaiser

Steve Rosenberg  
Mark Dudley  
Paul Robbins  
James Yang  
Rick Morgan

Dan Powell  
Keith Kerstann

Tom Waldmann